Program and Abstracts

BELTSVILLE SYMPOSIUM XX

Biotechnology's Role in the Genetic Improvement of Farm Animals

May 14-17, 1995

U.S. Department of Agriculture
Agricultural Research Service
Beltsville, Maryland

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United States Department of Agriculture
Agricultural Research Service
Beltsville Area Director's Office
Beltsville, Maryland 20705

WELCOME TO SYMPOSIUM PARTICIPANTS

Biotechnology is a new pillar of the agricultural system, and increasingly regarded as a key to U.S. agriculture's continuing success in today's expanding global economy. The predicted growth in the world's population will place tremendous pressures on agricultural production to mitigate hunger and malnutrition. This presents both a great challenge and an opportunity for animal biotechnology. After years of intensive effort and struggle, the movement of research discoveries from the laboratory to the livestock and poultry production stage is beginning to accelerate. Already there are biotechnological innovations having impact on animal disease diagnosis and treatment. Biotechnologies that enhance production efficiency are just now ready to market. These are tantalizing hints of the great promise of biotechnology in revolutionizing all aspects of livestock and poultry production, from genetics to reproduction to nutrition and product utilization.

The intent of this Symposium XX is to assess the progress of biotechnological research on the genetic improvement of farm animals, to share ideas and insights, to suggest solutions to technical bottlenecks, and to develop a shared vision. The political and social issues are an important part of these discussions, because without a full understanding of the social implications of the technologies produced, important and useful innovations may be strangled in a political environment poorly prepared for their introduction.

The organizers of this Symposium have succeeded in bringing together many of the world's authorities on biotechnology and farm policy to lead these discussions. The outcome of this meeting will be an important step in the evolution of a field that will play a vital role in tomorrow's global well-being.

K D Murrell

K. D. MURRELL
Director
Beltsville Area

TELEX 89491

ACKNOWLEDGMENTS

The **Friends of Agricultural Research-Beltsville (FAR-B), Inc.**, are cosponsors of the Beltsville Symposium series. FAR-B is a nonprofit group dedicated to supporting the research and educational programs at the Beltsville Agricultural Research Center. Membership is made up of former and current employees and a growing number of industry supporters. The Beltsville Symposium XX Committee thanks the members of FAR-B for their many contributions to the success of this symposium.

In addition to FAR-B, Inc., the Beltsville Symposium XX Organizing Committee also expresses its appreciation to the following organizations that provided financial contributions in support of the symposium:

Sponsors (donation of $500 or more as of April 15, 1995)

American Farm Bureau Federation, Park Ridge, IL
Avian Farms International, Inc., Waterville, ME
DeKalb Swine Breeders, Inc., DeKalb, IL
Monsanto, St. Louis, MO
Pig Improvement Corporation, Franklin, KY

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Hubbard Farms, Inc., Walpole, NH
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PREVIOUS BELTSVILLE SYMPOSIA

1976 Virology in Agriculture
1977 Biosystematics in Agriculture
1978 Animal Reproduction
1979 Human Nutrition Research
1980 Biological Control in Crop Production
1981 Strategies of Plant Reproduction
1982 Genetic Engineering: Applications to Agriculture
1983 Agricultural Chemicals of the Future
1984 Frontiers of Membrane Research
1985 Biotechnology for Solving Agricultural Problems
1986 Research Instrumentation for the 21st Century
1987 Biomechanisms Regulating Growth and Development: Keys to Progress
1988 Biotic Diversity and Germplasm Preservation-Global Imperatives
1989 The Rhizosphere and Plant Growth
1990 Remote Sensing for Agriculture
1991 Photomorphogenesis in Plants: Emerging Strategies for Crop Improvement
1992 Agricultural Water Quality Priorities
1993 Pest Management: Biologically Based Technologies
1994 Advances in Human Energy Metabolism

FOREWORD

The abstracts and biographical sketches in this program/abstract book were prepared for Beltsville Symposium XX, "Biotechnology's Role in the Genetic Improvement of Farm Animals." Presenting authors are indicated in the abstracts by all uppercase letters, and all authors are identified in the index by cross-references to abstract numbers.

The members of the Beltsville Symposium XX Organizing Committee included:

H.D. Norman (cochair), Animal Improvement Programs Laboratory, USDA-ARS
V.G. Pursel (cochair), Gene Evaluation and Mapping Laboratory, USDA-ARS
V.L. Hupfer (symposium secretary), Beltsville Area Office, USDA-ARS
M.R. Bakst (transportation), Germplasm and Gamete Physiology Laboratory, USDA-ARS
M. Beroza, Friends of Agricultural Research-Beltsville, Inc.
S.M. Hays (public relations), Information Staff, USDA-ARS
J.K. Lunney (finance), Parasite Immunobiology Laboratory, USDA-ARS
R.H. Miller (proceedings editor), Milk Secretion and Mastitis Laboratory, USDA-ARS
C.E. Rexroad, Jr. (registration), Gene Evaluation and Mapping Laboratory, USDA-ARS

Special appreciation is extended to **S.M. Hubbard**, Animal Improvement Programs Laboratory, USDA-ARS, for technical editing and formatting of the program/abstract book.

H. Duane Norman

H. Duane Norman
Cochair, Beltsville Symposium XX Organizing Committee

Vernon G. Pursel

Vernon G. Pursel
Cochair, Beltsville Symposium XX Organizing Committee

FAR-B DISTINGUISHED SCIENTIST AWARD

Harold W. Hawk

Dr. Hawk was born in Crawford County, PA, in 1927. He received a B.S. from Pennsylvania State University in 1952 and an M.S. and a Ph.D. from the University of Wisconsin in 1953 and 1956. Since 1956, he has conducted research in reproductive physiology at the USDA's Beltsville Agricultural Research Center. He served as Investigations Leader of the Physiology Investigations Unit (1964 to 1972) and as Chief and Research Leader (1972 to 1991) of the Reproduction Laboratory of the Animal Science Institute (later the Livestock and Poultry Sciences Institute). He retired from the Gene Evaluation and Mapping Laboratory in December 1993.

Dr. Hawk's research has generally been concerned with female fertility and function of the female reproductive tract and has included research with cattle, sheep, and small animals. These lines of research have included various aspects of dairy cattle reproduction; the hormonal control of various uterine functions, especially uterine defensive mechanisms, uterine contractility, and uterine composition; investigation of the physiological responses of the uterus to the presence of intrauterine contraceptive devices; problems of sperm survival and transport in the female reproductive tract, particularly after artificial regulation of the estrous cycle; and problems involved in the in vitro fertilization of bovine oocytes and culture of the embryos. He participated in research that produced the first sexed offspring from separated X and Y sperm. These various lines of research resulted in about 140 refereed journal articles and several dozen abstracts, review papers, and book chapters.

In 1982, Dr. Hawk initiated a major change in direction of research of the laboratory: a cooperative research project to produce animals with a foreign gene in their genome. Scientists in the laboratory were the first to produce and to test transgenic farm animals—animals that contain foreign gene constructs created by recombinant DNA technology. Since then, scientists of the laboratory have inserted more than 20 different gene and promotor combinations into sheep and swine zygotes and produced and tested hundreds of transgenic animals. These various investigations have demonstrated the potential for rapidly altering some of the production characteristics of farm animals. This pioneering work has elicited worldwide interest among researchers and the general public and has stimulated research in identifying and isolating desirable genes for transfer into farm animals.

Dr. Hawk received the USDA Superior Award in 1966, the Upjohn Lectureship from the American Fertility Society in 1968, and the Physiology and Endocrinology Award from the American Society of Animal Science in 1972; was named an Alumni Fellow of Pennsylvania State University in 1986; and received the USDA Distinguished Service Award in 1992.

GENERAL INFORMATION

Registration

Sun., May 14	5-8 pm	Holiday Inn, 10000 Baltimore Blvd. (intersection of Routes 1 and 95)
Mon., May 15	7:30 am-3 pm	Room 020, Bldg. 003, BARC-West (10300 Baltimore Ave., Route 1)
Tue., May 16	7:30 am-3 pm	Room 020, Bldg. 003, BARC-West
Wed., May 17	7:30-9 am	Room 020, Bldg. 003, BARC-West

Podium Presentations

All podium presentations will be given at the Beltsville Agricultural Research Center (BARC) in the auditorium of Bldg. 003, BARC-West.

Poster Presentations

All posters will be displayed in the grand ballroom of the Holiday Inn. Presentation of posters will be split into A (odd number) and B (even number) sessions. Posters may be set up and removed at the end of evening socials or from 7 to 7:45 am on Monday and Tuesday, May 15 and 16. All posters must be removed by 7:45 am on Wednesday, May 17.

Luncheons and Evening Socials

All luncheons and evening socials will be held in the grand ballroom of the Holiday Inn. Tickets for luncheons and beverages at evening socials are included in the registration packet.

Slide Preview

Podium presenters can preview their slides in Room 020, Bldg. 003, BARC-West.

Exhibits

Computers with Internet links for display of databases will be available in Room 020, Bldg. 003, BARC-West, on Monday and Tuesday, May 15-16, from 8 am to 5 pm. Commercial exhibits are on display in the Prince George's Room of the Holiday Inn.

Post Office

A symposium substation of the Beltsville Post Office will be in Room 020, Bldg. 003, BARC-West, Beltsville, MD, on Monday and Tuesday, May 15-16, from 9 am to 3:30 pm.. A special pictorial cancellation will be available. The proceeds from sales of commemorative envelopes will be donated to the Friends of Agricultural Research-Beltsville, Inc.

ARCEA

The Agricultural Research Center Employees' Association (ARCEA) will have souvenirs available for purchase in Room 020, Bldg. 003, BARC-West, Beltsville, MD, on Monday, May 15, from 9:45 am to 4:15 pm, on Tuesday, May 16, from 9:30 am to 3:30 pm, and on Wednesday, May 17, from 9:30 am to 1 pm.

SCHEDULE OF EVENTS

BELTSVILLE SYMPOSIUM XX
MAY 14-17, 1995

SUNDAY, MAY 14, 1995

Registration, poster setup, and opening social will be held at the Holiday Inn, 10000 Baltimore Blvd. (U.S. Routes 1 & 95), Beltsville, MD:

Registration	5-8 pm	Hallway in front of grand ballroom
Poster setup (Session A, odd numbers)	5-10 pm	Grand ballroom
Social	5:30-10 pm	Grand ballroom

Beverage tickets for the social are in the registration packet.

MONDAY, MAY 15, 1995

Opening Session (Auditorium, Bldg. 003, BARC-West)

8:15 am **Symposium Introduction**
Dr. H. Duane Norman, Animal Improvement Programs Laboratory, USDA-ARS, Beltsville, MD

8:20 am **Welcome**
Dr. K. Darwin Murrell, Director, Beltsville Area, USDA-ARS, Beltsville, MD

8:30 am **Presentation of FAR-B Distinguished Scientist Award to Dr. Harold Hawk**
Dr. George Irving, Friends of Agricultural Research-Beltsville, Inc., Beltsville, MD

8:40 am **KEYNOTE ADDRESS**
On the Evolution of Genome Organization in Mammals
Dr. Stephen O'Brien, Laboratory of Viral Carcinogenesis, National Cancer Institute, Frederick, MD

Session 1 Genetic Diversity (Auditorium, Bldg. 003, BARC-West)

9:25 am **Session Introduction**
Chair, Dr. Vernon Pursel, Gene Evaluation and Mapping Laboratory, USDA-ARS, Beltsville, MD

9:30 am **Implications of the Convention on Biological Diversity for Management of Domestic Animal Diversity**
Dr. Donald Plucknett, Agricultural Research and Development International, Annandale, VA

10:00 am **Break**

10:20 am **Exotic Germplasm: Finding a Use for These Genes Today**
Dr. Patrick Cunningham, Department of Genetics, Trinity College, University of Dublin, Dublin, Ireland

MONDAY, MAY 15, 1995

Session 1 Genetic Diversity (Auditorium, Bldg. 003, BARC-West)

10:50 am **The FAO Program for Management of Global Animal Genetic Resources**
Dr. Keith Hammond, Animal Production and Health Division, Food and Agriculture Organization, United Nations, Rome, Italy

11:20 am **Animal Germplasm Programs in North America**
Dr. Roger Gerrits, National Program Staff, USDA-ARS, Beltsville, MD

Luncheon and Poster Session A (Odd Numbers)
(Noon to 2 pm, Grand Ballroom, Holiday Inn, Beltsville, MD)

Luncheon tickets are in the registration packet. Presenters for poster session A will be available to discuss their research from 12:30 to 1:45 pm.

Session 2 Mapping Technologies (Auditorium, Bldg. 003, BARC-West)

2 pm **Session Introduction**
Chair, Dr. Caird Rexroad, Gene Evaluation and Mapping Laboratory, USDA-ARS, Beltsville, MD

2:05 pm **Development of Detailed Microsatellite Maps in Livestock**
Dr. Craig Beattie, U.S. Meat Animal Research Center, USDA-ARS, Clay Center, NE

2:50 pm **Chromosome-Specific Libraries and Use in Swine Genome Mapping**
Dr. Joan Lunney, Parasite Immunobiology Laboratory, USDA-ARS, Beltsville, MD

3:20 pm **Mapping and the Chicken: Expressed Sequence Tags and Genetic Regulatory Elements**
Dr. Susan Lamont, Department of Animal Science, Iowa State University, Ames, IA

3:50 pm **Break**

4:10 pm **The Map and Its Use in Sheep**
Dr. Noelle Muggli-Cockett, Department of Animal, Dairy, and Veterinary Sciences, Utah State University, Logan, UT

4:40 pm **Integrating the Syntenic, Physical, and Linkage Maps**
Dr. James Womack, Department of Veterinary Pathobiology, Texas A&M University, College Station, TX

5:10 pm **Mapping Genes for Disease Resistance: Lessons from the Bovine Leukemia Virus**
Dr. Harris Lewin, Laboratory of Immunogenetics, Department of Animal Sciences, University of Illinois, Urbana, IL

MONDAY, MAY 15, 1995

Evening Social and Continuation of Poster Session A (6 to 10 pm, Grand Ballroom, Holiday Inn, Beltsville, MD)

Beverage tickets are in the registration packet. Presenters for poster session A are encouraged to be available to discuss their research during the evening social. Posters from session A may be removed from 8:30 to 9 pm; presenters for poster session B may set up from 9 to 10 pm (or from 7 to 7:45 am on Tuesday, May 16).

TUESDAY, MAY 16, 1995

Session 3 Embryo & Gamete Technology (Auditorium, Bldg. 003, BARC-West)

8 am **Session Introduction**
Chair, Dr. Murray Bakst, Germplasm and Gamete Physiology Laboratory, USDA-ARS, Beltsville, MD

8:05 am **In Vitro Maturation and Fertilization of Pig Oocytes**
Dr. Billy Day, Department of Animal Sciences, University of Missouri, Columbia, MO

8:40 am **Factors Influencing Development of Embryos Produced by Nuclear Transfer**
Dr. Ian Wilmut, Department of Development and Reproduction, Roslin Institute, Roslin, Midlothian, United Kingdom

9:15 am **Gender Preselection in Mammals**
Dr. Larry Johnson, Germplasm and Gamete Physiology Laboratory, USDA-ARS, Beltsville, MD

9:50 am **Break**

10:10 am **Modification of Milk Composition in Transgenic Animals**
Dr. Robert Wall, Gene Evaluation and Mapping Laboratory, USDA-ARS, Beltsville, MD

10:45 am **Embryonic Stem Cells for Farm Animals**
Dr. Steven Stice, Advanced Cell Technology, Inc., Paige Laboratory, University of Massachusetts, Amherst, MA

11:20 am **Primordial Germ Cells for Genetic Modification of Poultry**
Dr. Bernard Wentworth, Department of Poultry Science, University of Wisconsin, Madison, WI

TUESDAY, MAY 16, 1995

Luncheon and Poster Session B (Even Numbers)
(Noon to 2 pm, Grand Ballroom, Holiday Inn, Beltsville, MD)

Luncheon tickets are in the registration packet. Presenters for poster session B will be available to discuss their research from 12:30 to 2 pm.

Session 4 Acceptance of Biotechnology: Political & Social Issues (Auditorium, Bldg. 003, BARC-West)

2:15 pm **Session Introduction**
Chair, Dr. Robert Zimbelman, American Society of Animal Science, Bethesda, MD

2:20 pm **Food and Drug Administration Concerns Regarding Genetic Improvement of Farm Animals**
Dr. Steven Bauer, Molecular Immunology Laboratory, Center for Biologics Evaluations and Research, FDA, Bethesda, MD

2:40 pm **Acceptance of Biotechnology: Food Safety and Inspection Service Concerns**
Dr. Pat Basu, Science and Technology, USDA-FSIS, Washington, DC

3:00 pm **Break**

3:20 pm **Public Perceptions of Animal Biotechnology**
Dr. Gerald Gaull, The Ceres Forum, The Center for Food and Nutrition Policy, Washington, DC

3:40 pm **Congressional Concerns Regarding Biotechnology**
Hon. Wayne Allard *(invited)*, Colorado 4th Congressional District, U.S. House of Representatives, Washington, DC

4:20 pm **Speakers Panel** (Question and Answer Session)

Evening Social and Continuation of Poster Session B
(7 to 11 pm, Grand Ballroom, Holiday Inn, Beltsville, MD)

Beverage tickets are in the registration packet. Presenters for poster session B are encouraged to be available to discuss their research during the evening social. Posters from session B may be removed from 9:30 to 10:30 pm. All posters must be removed by 7:45 am on Wednesday, May 17.

WEDNESDAY, MAY 17, 1995

Session 5 Marker Application (Auditorium, Bldg. 003, BARC-West)

8 am — **Session Introduction**
Chair, Dr. Joan Lunney, Parasite Immunobiology Laboratory, USDA-ARS, Beltsville, MD

8:05 am — **Introduction to QTL Detection and Marker-Assisted Selection**
Dr. Joel Weller, Animal Genetics and Breeding Unit, Institute of Animal Sciences, Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel

8:40 am — **Mapping Genes that Influence Meat Characteristics of Pigs**
Dr. Leif Andersson, Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden

9:15 am — **Improving Dairy Cattle Using Marker Data**
Dr. Margaret Dentine, Department of Dairy Science, University of Wisconsin, Madison, WI

9:50 am — **Break**

10:10 am — **The Use of Markers in Poultry Improvement**
Dr. Wayne Fairfull, Centre for Food and Animal Research, Agriculture Canada, Ottawa, ON, Canada

10:45 am — **Databases and Information Systems Needed for Maps and Marker-Assisted Selection**
Dr. John Keele, U.S. Meat Animal Research Center, USDA-ARS, Clay Center, NE

11:20 am — **Integrating Use of New Markers into the Real World**
Dr. Paul VanRaden, Animal Improvement Programs Laboratory, USDA-ARS, Beltsville, MD

Symposium Closing
(11:55 am, Auditorium, Bldg. 003, BARC-West)

Dr. H. Duane Norman, Animal Improvement Programs Laboratory, USDA-ARS, Beltsville, MD

PODIUM BIOGRAPHIES

BELTSVILLE SYMPOSIUM XX
MAY 14-17, 1995

1 DR. STEPHEN J. O'BRIEN

Geneticist
Laboratory of Viral Carcinogenesis, National Cancer Institute, Frederick, MD 21702-1201, USA

Dr. O'Brien is chief of the Laboratory of Viral Carcinogenesis at the National Cancer Institute. He received a B.S. in biology from St. Francis College (1966) and a Ph.D. in genetics at Cornell University (1971). He is the first or sole author of over 90 publications and a contributing author for over 300. He is a member of the American Genetic Association, Cat Specialist Group, International Union for the Conservation of Nature and Species Survival Commission, American Association for the Advancement of Science, Genetics Society of America, New York Academy of Science, and American Association of Zoological Parks and Aquariums. Dr. O'Brien is founder and co-director of the New Opportunities in Animal Health Sciences Center for Wildlife Sciences, Smithsonian Institution. He was elected fellow of the American Academy of Arts and Sciences and received the Distinguished Alumnus in Natural Sciences Award from St. Francis College. He is executive editor of the *Journal of Heredity* and associate editor of *Mammalian Genetics* and *Molecular Phylogenetics and Evolution.* Dr. O'Brien serves as adjunct professor at several universities. He is chair of the International Committee on Comparative Gene Mapping and the editor of *Genetic Maps*. His research interests include molecular genetics, viral oncology, immunology and reproductive physiology, molecular evolution, and population genetics.

2 DR. DONALD L. PLUCKNETT

President
Agricultural Research and Development International, Annandale, VA 22003, USA

A native of Nebraska, Dr. Plucknett received a B.S. and an M.S. at the University of Nebraska and a Ph.D. in tropical soil science at the University of Hawaii. He spent more than 20 years in Hawaii as a professor of agronomy and soil science, and his experience in international agriculture began there. From 1973 to 1976 and from 1978 to 1980, while on loan from the University of Hawaii, he held positions with the U.S. Agency for International Development as chief of Soil and Water Management of the Technical Assistance Bureau, deputy executive director of the Board for Food and Agricultural Development, and chief of Agriculture and Rural Development in the Asia Bureau. He joined the World Bank in 1980 as senior science advisor to the Consultative Group on International Agriculture Research and served until retirement in September 1993. He has written some 20 books and more than 200 articles, including *Genebanks and the World's Food*, *Tropical Forests and Their Crops*, and *Networking in International Agricultural Research.* He is a fellow of the American Society of Agronomy, Soil Science Society of America, Crop Science Society of America, American Association for the Advancement of Science, and the Linnean Society of London. He is a vocal proponent of the importance of the management, availability, and use of genetic resources as a foundation for agricultural improvement and to meet world food needs.

3 DR. PATRICK CUNNINGHAM

Professor of animal genetics
Genetics Department, Trinity College, University of Dublin, Dublin 2, Ireland

Dr. Cunningham received a B.S. in agriculture and an M.S. in animal nutrition from the National University of Ireland. After receiving a Ph.D. in animal breeding and genetics from Cornell University in 1962, he served as the senior research officer and head of the Animal Breeding and Genetics Department and later as deputy director of the Irish National Agricultural and Food Research Institute at Trinity College (1962 to 1988). Since 1974, he has been a professor of animal genetics at Trinity College, Dublin. Dr. Cunningham also was a visiting professor at the Agricultural University of Norway (1968 to 1969) and the Economic Development Institute of the World Bank (1988). He was director of the Animal Production and Health Division of the Food and Agriculture Organization in Rome (1990 to 1993) and the Screwworm Emergency Centre for North Africa (1990 to 1992). Dr. Cunningham is the author of over 100 publications in the areas of selection and crossbreeding theory, economic aspects of genetic improvement (particularly for cattle populations), genetic analysis of horse populations, and economic development and molecular evolution of cattle populations.

4 DR. KEITH HAMMOND

Senior officer
Animal Genetic Resources Group, Animal Production and Health Division, Food and Agriculture Organization of the United Nations, 00100 Rome, Italy

Dr. Hammond, an Australian of farming background, received a Ph.D. in quantitative genetics from the University of Sydney while a trainee with a state Department of Agriculture. A 1974 Churchill fellow, he was invited to deliver a lecture series on animal genetics in 1975 at the University of New England (UNE), Armidale, New South Wales, Australia. In 1976, he initiated a research institute, the Animal Genetics and Breeding Unit (AGBU), as a hybrid operation involving both UNE and the Australian Department of Agriculture to do research primarily for Australian livestock industries. Dr. Hammond directed AGBU for a number of years before being awarded a personal chair in animal science at UNE. In 1993, he accepted the post of senior officer of the Animal Genetic Resources Group with the United Nations' Food and Agriculture Organization with a mandate to design, to help generate the funding for, and to implement a global program for the better management of the genetic resources for the animal species of interest to food and agriculture.

5 **DR. ROGER J. GERRITS**
National program leader (animal production and germplasm)
National Program Staff, USDA-ARS, Beltsville, MD 20705-2350, USA

Dr. Gerrits was born on a livestock farm in 1932 in Green Bay, WI. He completed a B.S. at the University of Wisconsin in 1958 and received an M.S. (1960) and a Ph.D. (1963) from the University of Minnesota. Dr. Gerrits joined USDA-ARS in 1963. He served as physiology research leader, chief of the Swine Research Branch, and national program leader (NPL) for swine production and diseases and agricultural chemicals. He currently is NPL for animal production and germplasm. He has provided leadership for numerous pioneering research efforts that have advanced knowledge of animal biology and improved efficiency of animal agriculture, including estrus synchronization, frozen semen, transgenic swine, gender preselection, importation of Chinese swine, and mapping livestock and poultry genomes. He has been an active leader with university scientists and commodity groups, especially the swine industry. He represented the United States in development of the International Animal Genetic Resources Program and is vice president of the World Association for Animal Production. He has received several awards from industry, government, and academia, including USDA's Superior Service Award and the National Pork Producers Council's Distinguished Service Award. In 1994, he received the American Society of Animal Science's Animal Industry Award. Dr. Gerrits is an author of over 100 publications on animal agriculture.

6 **DR. CRAIG W. BEATTIE**
Research chemist
U.S. Meat Animal Research Center, USDA-ARS, Clay Center, NE 68933-0166, USA

Dr. Beattie received a B.S. and M.S. in biological sciences from Fairleigh Dickinson University and a Ph.D. in biological sciences from the University of Delaware. A postgraduate fellowship in pharmacology at the University of Illinois School of Medicine followed. After several years as a research group leader at the Wyeth Institute, Wyeth Laboratories, Dr. Beattie joined the Department of Pharmacology, Bowman Gray School of Medicine and subsequently the Division of Surgical Oncology, Department of Surgery, University of Illinois. At Illinois, Dr. Beattie was also tenured in the Departments of Pharmacology and Medicinal Chemistry. He joined the Roman L. Hruska U.S. Meat Animal Research Center, Agricultural Research Service, USDA, as group leader for genome mapping and expression in 1991. Dr. Beattie has also held visiting professorships at the University of New South Wales and Sydney University, Sydney, Australia, and currently holds adjunct professorships in the Department of Surgical Oncology, University of Illinois; Physiology and Pharmacology, College of Veterinary Medicine, Texas A&M University; and Department of Veterinary PathoBiology, College of Veterinary Medicine, University of Minnesota. His research is currently focused on the development of comprehensive genomic maps for livestock and the genetics of disease resistance.

7 **DR. JOAN K. LUNNEY**
Acting research leader and supervisory research immunogeneticist
Parasite Immunbiology Laboratory, USDA-ARS, Beltsville, MD 20705-2350, USA

Dr. Lunney received a B.S. in chemistry in 1968 from Chestnut Hill College, Philadelphia, PA. She taught chemistry at Camden Catholic High School in New Jersey until 1971 when she started a Ph.D. in biochemistry at Johns Hopkins University in Baltimore, MD. Her Ph.D. research on glycoprotein receptors was performed with Dr. Gilbert Ashwell at the National Institute of Health (NIH). For postdoctoral research, Dr. Lunney moved in 1976 to the Immunology Branch of NIH's National Cancer Institute and started work with Dr. David Sachs on pig major histocompatibility complex antigens and swine cellular immunology. In 1983, she joined the Helminthic Diseases Laboratory, USDA-ARS, and started analyzing immunogenetic control of swine responses to the parasitic diseases trichinellosis and toxoplasmosis. Currently Dr. Lunney serves on advisory/editorial boards for *Animal Genetics*, *Animal Biotechnology*, and *Veterinary Immunology and Immunopathology*. She was guest editor of the *Veterinary Immunology and Immunopathology* special issue on porcine immunology that also summarized the Swine CD Workshop, which she chaired. Dr. Lunney was selected as a member of the National Research Council's Institute of Medicine Committee on Xenograft Transplantation. She is a coauthor of the plan for the USDA National Animal Genome Research Program and currently serves as chair of its Swine Species Committee and the NC-210 project on mapping the pig genome.

8 **DR. SUSAN J. LAMONT**
Professor of animal science
Department of Animal Science, Iowa State University, Ames, IA 50011-3150, USA

Dr. Lamont received a Ph.D. from the University of Illinois Medical Center in 1980 after completing dissertation research on avian immunology. She spent 3 years as a postdoctoral research associate in the Veterinary and Animal Sciences Department, University of Massachusetts, where she studied immunological aspects of a chicken genetic model for a human disease. In 1983, she joined the faculty of the Department of Animal Science at Iowa State University where she is now a professor and also half-time assistant director (biological sciences) of the Iowa Agriculture Experiment Station. Her research at Iowa State University has focused on genetic regulation of immune response and disease resistance of chickens. More recently, projects on avian molecular genetics, including gene mapping and identification of molecular markers for biological traits, have been conducted. Dr. Lamont teaches graduate courses on poultry breeding and genetics and on livestock immunogenetics. She also is currently serving as the secretary of the International Society of Animal Genetics, a director of the World Poultry Science Association, and the chair of the U.S. National Animal Genome Technical Committee.

9 **DR. NOELLE E. MUGGLI-COCKETT**

Associate professor
Department of Animal, Dairy, and Veterinary Sciences, Utah State University, Logan, UT 84322-4700, USA

Dr. Muggli-Cockett received an M.S. and Ph.D. in animal breeding and genetics from Oregon State University. After completing her degrees, she was a research geneticist at the U.S. Meat Animal Research Center, USDA-ARS, in Clay Center, NE, until she joined the Department of Animal, Dairy, and Veterinary Sciences at Utah State University as an assistant professor in 1990. She was promoted to associate professor in 1993. She currently teaches principles of animal breeding, practical animal breeding, and biotechnology in agriculture. Dr. Muggli-Cockett's current research program is focused on characterization of the callipyge gene causing muscle hypertrophy of sheep and identification of a genetic marker for spider lamb syndrome of sheep. She has published 22 refereed journal articles, 35 abstracts, 10 technical reports, and 31 invited proceedings. During the last 5 years, she has been principal investigator of 23 competitive research and teaching grants, with a total of $1,153,730 awarded in funding. Dr. Muggli-Cockett serves as the section editor for the "Molecular Genetic Markers" section of the *Journal of Animal Science*, is the secretary of the BoLA Nomenclature Committee, and acts as the National Animal Genome Research Program's sheep genome mapping coordinator. She recently was asked to serve as the North American editor for *Animal Genetics*. She serves on a number of committees at Utah State University.

10 **DR. JAMES E. WOMACK**

W.P. Luse endowed professor
Department of Veterinary Pathobiology, Texas A&M University, College Station, TX 77843-4467, USA

Dr. Womack received a B.S. in mathematics from Abilene Christian College in 1964 and a Ph.D. in genetics from Oregon State University in 1968. Before joining the faculty of Texas A&M University in 1977, he was a staff scientist at the Jackson Laboratory, Bar Harbor, ME, where he initiated studies in comparative mapping of the genomes of mice and humans. He began developing a gene map of domestic cattle at Texas A&M University in 1982, a work that has continued to the present. He received the 1993/94 CIBA prize for research in animal health for his pioneering work in the development of a map of the bovine genome. Dr. Womack is the cattle coordinator for the National Animal Genome Research Program and a member of the Human Genome Organization; he serves on the editorial boards of the *Journal of Heredity*, *Animal Biotechnology*, *Mammalian Genome*, and *Genomics*. He has published more than 150 full-length publications.

11 **DR. HARRIS A. LEWIN**

Professor of immunogenetics
Department of Animal Sciences, University of Illinois at Urbana-Champaign, Urbana, IL, 61801, USA

Undergraduate and graduate studies in animal breeding and genetics earned Dr. Lewin a B.S. (1979) and an M.S. (1981) from Cornell University. In 1984, he received a Ph.D. in immunology from the University of California at Davis. Dr. Lewin then joined the faculty of the Department of Animal Sciences at the University of Illinois as an assistant professor of immunogenetics. In 1989, he was promoted to associate professor and, in 1994 to professor. Dr. Lewin's research program has focused on elucidation of the bovine major histocompatibility complex, host genetic mechanisms of resistance to bovine leukemia virus infection and disease progression, gene mapping, and mapping of economic trait loci of cattle. Dr. Lewin has published more than 100 book chapters, peer-reviewed journal articles, and technical reports and has given invited lectures and symposia in 11 countries. He serves as editor of the National Animal Genome Research Program (NAGRP) newsletter and associate editor of *Animal Biotechnology*. Among other service activities, Dr. Lewin was chair of the NAGRP Cattle Genome Coordinating Committee and cochair and organizer of the Conference on Comparative Genetics of Terrestrial and Aquatic Vertebrates (1994), Oslo, Norway. He currently serves as chair of the Dairy Bull DNA Repository Steering Committee. Dr. Lewin received the College of Agriculture's Young Faculty Award for Excellence in Research in 1992 and was recognized as a University Scholar in 1993.

12 **DR. BILLY N. DAY**

Professor
Department of Animal Sciences, University of Missouri, Columbia, MO 65211, USA

Dr. Day was born in Arthur, WV. He attended West Virginia University and received a B.S. in 1952 and an M.S. in 1954. He was awarded a Ph.D. in 1958 at Iowa State University under the direction of Dr. Robert M. Melampy. Dr. Day joined the faculty of the University of Missouri as an assistant professor in 1958 and assumed leadership of the research program in reproductive physiology of swine in the Department of Animal Husbandry. His sabbatical leave in 1965 was with Dr. C. Polge at the ARC Unit of Reproductive Physiology and Biochemistry, Cambridge, England. Dr. Day has pioneered basic and applied research physiology of reproduction, artificial insemination, and embryonic development. He also has developed successful embryo culture and transfer techniques for swine. His program is presently giving emphasis to fertilization, culture, and micromanipulation of early porcine embryos. Transfer of fused embryos to recipient animals have resulted in the birth of chimeric pigs. In a related study, successful in vitro maturation and fertilization of swine embryos has been achieved as measured by embryonic and fetal development. Dr. Day has had an active graduate and postdoctorate training program. He is a dedicated scientist who has worked toward the improvement of reproductive efficiency in farm animals with emphasis on swine reproduction. Dr. Day and his wife, Annabelle, have three sons, two daughters, and five grandchildren.

13 **DR. IAN WILMUT**

Mammalian embryologist
Department of Development and Reproduction, Roslin Institute, Roslin, Midlothian EH25 9PS, United Kingdom

Dr. Wilmut is the head of a laboratory concerned with understanding the mechanisms that regulate early mammalian development and using that knowledge to establish novel methods of animal breeding and production. Current projects are investigating factors that influence the maintenance of plutipotent cells in culture, factors that influence development after nuclear transfer, the role of growth factors in bovine embryo development, and the cause of unusually large fetuses following embryo culture or manipulation. After gaining a B.S. at the University of Nottingham under Professor Lamming, Dr. Wilmut carried out research for a Ph.D. with Professor Polge at Cambridge. His studies on the preservation of boar semen led naturally to research on embryo freezing in the same laboratory. At this time, the first calf was born from a frozen embryo. After moving to Edinburgh, Dr. Wilmut became interested in causes of prenatal wastage. This work led to the hypothesis that much natural wastage reflects physiological variation in the mechanisms that regulate embryonic development, the maternal environment, or the relationship between them. More recently, he was one of the group that produced human protein needed for the treatment of disease in the mammary gland of sheep. This project led to the current interest in developing novel methods for embryo production and manipulation.

14 **DR. LAWRENCE A. JOHNSON**

Research leader
Germplasm and Gamete Physiology Laboratory, USDA-ARS, Beltsville, MD 20705-2350, USA

Raised on a Wisconsin livestock farm, Dr. Johnson received a B.S. in agricultural education and chemistry from the University of Wisconsin at River Falls in 1961, an M.S. from the University of Minnesota in 1963, and a Ph.D. in animal science (physiology) from the University of Maryland in 1968. He joined ARS in 1964, first with the Swine Research Branch and then the Reproduction Laboratory. He became research leader of the Germplasm and Gamete Physiology Laboratory when it was created in 1991. Dr. Johnson is widely recognized as an international authority on semen physiology and preservation for livestock. His more recent research, the development of an effective method to control the sex ratio of mammalian offspring (Beltsville Sperm Sexing Technology) has brought him recognition as a world authority on gender preselection. Dr. Johnson is the recipient of a USDA Superior Service Award (1977), the T.W. Edminster Award from ARS (1988), the Animal Physiology and Endocrinology Award from the American Society of Animal Science (1991), the ARS Distinguished Research Scientist Award (1994), and the Alexander von Humboldt Foundation Award for 1994.

15 **DR. ROBERT J. WALL**

Research physiologist (animal)
Gene Evaluation and Mapping Laboratory, USDA-ARS, Beltsville, MD 20705-2350, USA

Upon receiving a B.S. in electrical engineering from Antioch College, Dr. Wall joined Lockheed Missiles and Space Company, Sunnyvale, CA, as a project advisor for the Poseidon missile program. In 1969, he moved to New Mexico to become the National Aeronautics and Space Agency's (NASA's) primary test conductor and electrical engineer of the altitude simulation system for testing the descent-stage engine of the lunar excursion module. While with NASA, Dr. Wall became owner-operator of a dairy goat farm and was elected director of the American Dairy Goat Association (ADGA) representing the southwestern United States. He also chaired ADGA's Artificial Insemination Committee. After attending New Mexico State University, Dr. Wall was granted an M.S. in zoology and cell physiology. He attended Cornell University and received a Ph.D. in physiology and biochemistry in 1981. He then joined ARS and initiated a research program to develop new strategies for identifying fertilization potential of bull sperm. At ARS, Dr. Wall became a member of the team that produced the first transgenic farm animals. His current research includes developing methods for improving efficiency of producing transgenic animals and exploring potential uses of transgenic livestock, with special emphasis on altering genetic control of mammary glands to improve milk quality and to create new livestock products. Dr. Wall has written numerous reviews and scientific abstracts and has authored or coauthored 59 peer-reviewed scientific journal articles.

16 **DR. STEVEN L. STICE**

Scientist
Advanced Cell Technology, Inc., Paige Lab, University of Massachusetts, Amherst, MA 01003, USA

Dr. Stice received a B.S. in agricultural science from the University of Illinois in 1983. He then attended Iowa State University and received an M.S. under the direction of Dr. Stephen Ford. While attending the University of Massachusetts, Dr. Stice completed his Ph.D. program guided by Dr. James Robl in 1989. During this time, Dr. Stice developed rabbit nuclear transfer procedures and published the first article on rabbit nuclear transfer offspring derived from blastomere donor nuclei. In 1988, Dr. Stice began working with TSI, Corp., in Worcester, MA. While at TSI, Corp., Dr. Stice was awarded two small business innovative research phase I grants. Upon completion of his Ph.D., Dr. Stice accepted a position as a research scientist at ABS Specialty Genetics, a research arm of American Breeders Service. At ABS Specialty Genetics, he refined bovine nuclear transfer procedures and initiated an embryonic stem cell program. This program produced five patent applications and several published manuscripts. In 1993, Dr. Stice became manager of cloning and stem cell research at ABS. Presently, he is a founding scientist at Advanced Cell Technology, Inc., a start-up biotechnology company with research emphasis on genetic modification in poultry, swine, and cattle.

17 DR. BERNARD C. WENTWORTH

Professor
Department of Poultry Science, University of Wisconsin, Madison, WI 53706-1284, USA

Dr. Wentworth received a B.S. at the University of Maine and an M.S. and a Ph.D. at the University of Massachusetts. His initial employment was with the Massachusetts Cooperative Wildlife Research Unit. He relocated to the University of Wisconsin in 1969 as an associate professor, was promoted to professor in 1975, and now serves as chair of the Department of Poultry Science. His research has focused on the biology of turkey reproduction with major management contributions in artificial insemination, lighting of breeder toms, and silage feeding of breeder hens and toms. He contributed to endocrine profiles of luteinizing hormone, prolactin, estradiol, progesterone, and testosterone. Dr. Wentworth has recently coordinated work in his laboratory to provide a working model for a non-viral method of gene transfer in birds by use of the primordial germ cell as a vehicle for germline modifications. He has taught courses in avian physiology, incubation and hatchery management, meat bird management, and biology and appreciation of companion animals as well as presented many guest lectures and seminars. He is on the graduate faculty in the Endocrinology-Reproductive Physiology Program. He has served as chair of the all-campus and college Animal Care Committee, Academic Planning Council, and Biological Divisional Committee. Dr. Wentworth is a retired colonel of the U.S. Army Medical Service Corps.

18 DR. STEVEN R. BAUER

Senior investigator
Laboratory of Molecular Immunology,
Division of Cellular and Gene Therapies,
Office of Therapeutics, FDA-CBER, Bethesda, MD 20892, USA

Dr. Bauer received a Ph.D. in biochemistry from the University of Maryland in 1986. His thesis work was done in the laboratory of Dr. Michael Potter at the National Cancer Institute and involved an investigation of the mutations responsible for oncogene dysregulation in mouse tumors of B-lymphocyte origin. He then became a scientific member of the Basel Institute for Immunology in Basel, Switzerland, from 1986 though 1991. In 1991, Dr. Bauer joined the Food and Drug Administration (FDA) Center for Biologics Evaluation and Research. As a member of the Division of Cellular and Gene Therapies, his current research relies on a transgenic model system developed in Basel and utilizes oncogene transgenic mice in the study of B-lymphocyte differentiation and neoplasia. Dr. Bauer currently is on the board of editors of *Transgenics*. Knowledge gained in his research and editorial capacities has served him well as cochair of an intercenter FDA committee convened to produce a "points to consider" document covering the manufacture and testing of therapeutic products for human use derived from transgenic animals.

19 DR. PAT BASU

Director
Technology Transfer and Coordination Staff, Science and Technology, USDA-FSIS, Washington, DC 20250, USA

Dr. Basu has a D.V.M. from Calcutta University (1967), an M.S. in pharmacology and toxicology from Ohio State University (1972), and graduate training in microbiology, epidemiology, and biotechnology at the University of Georgia, (1985). Prior to joining the Food Science and Inspection Service (FSIS) in 1982, he served as a supervisory veterinarian for the West Virginia Department of Agriculture for 9 years. He has also spent a number of years in private practice. With FSIS, Dr. Basu has worked in various supervisory and management positions. The FSIS is responsible for assuring the wholesomeness and safety of the nation's domestic and imported meat and poultry supply. The mission of the Technology Transfer and Coordination Staff (TTCS) is to promote the concept of constant modernization in the inspection program and to help bridge the gap between new developments in science and technology and the inspection mission of FSIS. As the director of TTCS, Dr. Basu is responsible for the development and implementation of all policies in FSIS involving biotechnology and transgenic animals. Dr. Basu has authored numerous scientific and regulatory papers and has received numerous awards and academic honors throughout his career, including USDA's Superior Service Award.

20 DR. GERALD E. GAULL

Director
The Ceres Forum, The Center for Food and Nutrition Policy, Washington, DC 20007-2197, USA

Dr. Gaull is the founding director of the Center for Food and Nutrition Policy and The Ceres Forum at Georgetown University. The Center seeks to improve communication and understanding of food and nutrition policy in areas such as biotechnology, food fortification, and nutrition education. The Ceres Forum provides continuing and direct discussion between leaders of the global food system and leaders of the public sectors affecting its operation. These programs are affiliated with Georgetown's Graduate Program in Public Policy, for which Dr. Gaull is a research professor of food policy. Prior to his present position, Dr. Gaull was vice president for nutritional science at the NutraSweet Company (1984 to 1992), where he directed strategy and policy for corporate nutritional scientific issues in governmental affairs, public relations, media, marketing, and sales. From 1965 to 1984, Dr. Gaull held various full-time faculty positions at Harvard University, Columbia University, and Mt. Sinai Medical School and adjunct professorships at Rockefeller University, the University of Chicago, and Northwestern University. Dr. Gaull is author/coauthor of over 200 scientific articles on nutrition, genetics, and developmental biochemistry in peer-reviewed professional journals and editor/coeditor of 7 books, including *New Technologies and the Future of Food Nutrition* (1991) and *The Emerging Global Food System: Public and Private Sector Issues* (1993).

21 HON. WAYNE ALLARD

Representative, Colorado 4th District
U.S. House of Representatives, Washington, DC 20515, USA

Rep. Wayne Allard is a successful small businessman who started his veterinary practice from scratch in Loveland, CO. He and his wife, Joan, have been married for more than 27 years, and they have two married daughters. After having worked exclusively in the private sector for 20 years, Rep. Allard was first elected to public office in 1982. He served in the Colorado State Senate for 8 years. During that time, he rose in the leadership ranks and was known for his change agenda. His most notable accomplishment was a limit on the length of the Colorado legislative session. In 1991, Rep. Allard was first sworn into the U.S. House of Representatives for Colorado's 4th District. He has pushed for change in the U.S. House by introducing legislation calling for Congress "to live under the same laws as everyone else." Rep. Allard also supports term limits and a Constitutional balanced budget amendment. Currently, Rep. Allard serves on the Budget Committee and the Agriculture Committee. He is chair of the House Agriculture Committee's Subcommittee on Resource Conservation, Research, and Forestry. He is also a member of the Resources Committee. During his two terms in Congress, his voting attendance has been better than 99%. He has also held more than 180 town meetings in the sprawling 21-county 4th District. He is a fiscal conservative, even in the management of his own office. In his two terms in Congress, Rep. Allard returned, unused, to the U.S. Treasury, more than $1 million in office funds.

22 DR. JOEL I. WELLER

Research scientist
Animal Genetics and Breeding Unit, Institute of Animal Sciences, Agricultural Research Organization, The Volcani Center, Bet Dagan 50250, Israel

Dr. Weller was born and grew up in Chicago, IL, and immigrated to Israel after graduation from high school. He received his B.S., M.S., and Ph.D. degrees from the Hebrew University of Jerusalem under the supervision of Dr. M. Soller. In 1976, Dr. Weller spent 6 months in the Israeli army. In 1982, Dr. Weller conducted postdoctoral research for 8 months at Cornell University, Ithaca, NY, with Dr. R.L. Quaas on multitrait genetic analysis via the reduced animal model and then for 1 year at the Animal Improvement Programs Laboratory, USDA-ARS, Beltsville, MD, on multitrait genetic analysis of milk production traits. Dr. Weller began working at Israel's Agricultural Research Organization in 1983 and received tenure in 1986. In 1987, he received a George A. Miller visiting scholar fellowship and spent 10 months at the University of Illinois, Urbana, working with Dr. D. Gianola on genetic analysis of categorical traits. Dr. Weller received the 1991 *Mashove* Man of the Year Award for his contribution to Israeli agriculture. In 1993, he returned to Beltsville for a 1-year sabbatical to study methods to locate individual loci that affect quantitative traits of dairy cattle. Dr. Weller lives in Rehovot, Israel, with his wife, Hedva, and four children.

23 DR. LEIF ANDERSSON

Professor of animal genetics
Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala Biomedical Center, S-751 24 Uppsala, Sweden

Dr. Andersson received a B.S. in biology from the University of Stockholm. He started his scientific career by carrying out population genetic studies of fish. He then moved to the Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, in Uppsala, where he received a Ph.D. in 1983 for studies on gene mapping of domestic animals with special reference to the horse. Since completion of his thesis, Dr. Andersson has been responsible for a program on molecular genetic research for domestic animals. One of the major projects has been characterization of major histocompatibility complex genes, including their evolution, polymorphism, and functional significance. The other major project is an extensive gene mapping program for pigs, which involves a cross between wild and domestic pigs. The program has involved the establishment of a linkage map, comparative mapping, and mapping of QTL. Dr. Andersson is a member of the International Society for Animal Genetics and the American Genetic Association. He is on the editorial boards for *Animal Genetics*, *Journal of Heredity*, and *Immunogenetics*.

24 DR. MARGARET R. DENTINE

Associate professor
Department of Dairy Science, University of Wisconsin, Madison, WI 53706, USA

Dr. Dentine received a B.S. from the University of California at Santa Cruz and an M.S. and a Ph.D. from North Carolina State University with graduate advisor Dr. Ben McDaniel. Upon graduation, she took a position as assistant professor with the Dairy Science Department at the University of Wisconsin. Her research areas have included evaluation for herdlife traits, young bull sampling programs, and marker-assisted selection of dairy cattle. Present research efforts include estimating effects of loci on performance traits, developing analysis methods for mapping loci involved in performance, and evaluating strategies for use of molecular data in selection schemes. She teaches classes on livestock breeding, selection experiments, and biotechnology in genetic improvement.

25 **DR. R. WAYNE FAIRFULL**

Senior research scientist
Centre for Food and Animal Research, Agriculture Canada, Ottawa, ON K0A 1B0, Canada

Dr. Fairfull was born and raised in Guelph, ON, Canada. He attended the University of Guelph and graduated with a B.S. in honors genetics. Remaining at Guelph, he completed an M.S. by carrying out a comparison of selection methods and mating plans using *Tribolium castaneum*. He received his Ph.D. from Dalhousie University in Halifax, NS, Canada, where he studied genetics of oysters and lobsters under culture conditions. Since 1978, Dr. Fairfull has worked in Ottawa with the Research Branch of Agriculture Canada and studied the breeding and genetics of chickens. His research has encompassed the study of heterosis and crossing, data recording systems, genetics of aging, disease genetics and eradication, behavior, management, genetics of eggshell strength, and cryopreservation. Recently, he has been part of a team that used embryonic nuclear magnetic resonance spectroscopy to predict lean growth of broilers. He has collaborated with scientists at the Centre for Food and Animal Research and at various universities on studies that have used molecular techniques to assess inbreeding, to predict heterosis, to study endogenous viral genes and their relationship to production and disease incidence, and to search for genes affecting quantitative traits. Dr. Fairfull, his wife, Marilyn, and their daughter, Megan, live on a small farm outside Ottawa, where they enjoy country life, gardening, and horseback riding.

26 **DR. JOHN W. KEELE**

Research animal scientist
U.S. Meat Animal Research Center, USDA-ARS, Clay Center, NE 68933-0166, USA

Dr. Keele received a B.S. and an M.S. from the University of Idaho and a Ph.D. from Ohio State University. His M.S. thesis was on ruminant protein nutrition, and his Ph.D. dissertation was on genetic variance component estimation. After receiving his Ph.D., he was a postdoctoral scientist at the University of Nebraska, where he evaluated genetic selection criteria for swine. When he first joined the scientific staff at the Roman L. Hruska U.S. Meat Animal Research Center (MARC), his efforts focused on computer modeling of growth and body composition of beef cattle. Since the genome mapping project was initiated at MARC, his efforts have focused on mathematical aspects of genome mapping. His work includes database design and implementation, resource population design, linkage analysis, complex segregation analysis, and prediction of response to marker-assisted selection. His main interest is in developing technology either to improve the quality of beef, pork, and lamb or to reduce the cost of producing it.

27 **DR. PAUL M. VanRADEN**

Research geneticist (animal)
Animal Improvement Programs Laboratory, USDA-ARS, Beltsville, MD, 20705-2350, USA

A former Dairy Herd Improvement supervisor, Dr. VanRaden received a B.S. in dairy science from the University of Illinois. After completing a marketing internship with Select Sires, an Ohio-based artificial breeding organization, he attended Iowa State University and received an M.S. and a Ph.D. in animal breeding. His doctoral research on estimation of variance components received awards from the American Dairy Science Association and Iowa State University. After completion of his graduate studies, Dr. VanRaden continued his work on genetic evaluation of dairy cattle as a postdoctoral researcher at Iowa State University and the University of Wisconsin. While at the University of Wisconsin, he developed a new set of genetic evaluation programs for Holsteins. The Holstein Association USA used these programs to produce its national sire and cow rankings for conformation traits from 1988 to 1992. After joining USDA, Dr. VanRaden found methods to simplify and to improve the animal model and then led the project to evaluate bulls for longevity, somatic cell score, and the net merit economic index. His research on genetic markers has included estimation methods and involvement in two ongoing projects to measure allele differences of dairy cattle.

PODIUM ABSTRACTS

BELTSVILLE SYMPOSIUM XX
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1 On the evolution of genome organization in mammals

S.J. O'BRIEN, Laboratory of Viral Carcinogenesis, National Cancer Institute, Frederick, MD

The scientific community has witnessed a recent explosion of gene mapping for a variety of mammalian organisms. The advancement of automation, microsatellite technology, and the use of efficient breeding schemes has allowed the genomes of diverse species of mammals to become quickly and efficiently saturated with coding genes, but more profusely with highly polymorphic DNA markers. These highly polymorphic markers are ideal for investigations of economically important traits, which are vital to agricultural species. However, genetic mapping of diverse genomes has also supported the investigation of the genome itself. Comparisons of sequence and mapping data produced by the livestock genome projects have enhanced the knowledge of genomic arrangement. By piecing together the data from genome projects for different species, merging both type I (coding genes) and type II (anonymous DNA) markers, scientists may now envision the process of genomic evolution. This knowledge will aid in the dissection of the biology of these diverse species, revealing the significance of genomic rearrangement and sequence divergence on physiology, disease processes, and speciation.

2 Implications of the Convention on Biological Diversity for management of domestic animal diversity

M.S. Strauss, American Association for the Advancement of Science, Washington, DC, and **D.L. PLUCKNETT**, Agricultural Research and Development International, Annandale, VA

The Convention on Biological Diversity emerged in 1992 as a broad international consensus about the need for global, regional, and national efforts to conserve ecosystems as well as plant, animal, and microbial species. The Convention calls for establishing plans and mechanisms to promote biological diversity conservation and for financial and technological assistance to nations with resources insufficient to implement such plans. In contrast to many earlier discussions of conservation is the Convention's reaffirmation of an individual nation's sovereignty over the resources within its borders. As a global effort to manage and conserve domestic livestock and poultry genetic resources has begun to emerge, understanding of the implications of the Convention for these efforts has been important. Although not explicitly noted in the Convention, livestock or poultry resources are implicitly part of its calls for national conservation activities and for the sustainable use of biological diversity.

3 Exotic germplasm: finding a use for these genes today

E.P. CUNNINGHAM, Department of Genetics, Trinity College, University of Dublin, Dublin, Ireland

Surprisingly few species of animals (and plants) are used in today's agriculture. However, the great variety of contexts to which these species must respond calls for an enormous range of genetic diversity within each species, and a spectrum of interaction between genotypes and environments exists. Genetic diversity now can be documented at the DNA level. The use of mitochondrial and Y-chromosome sequences, together with microsatellite allele frequencies, enables measurement of genetic variability within and between populations with considerable precision as well as facilitates measurement of gene flow between populations. Recent work on global diversity of cattle illustrates the use of these biotechnological techniques.

4 The FAO program for management of global animal genetic resources

K. HAMMOND, Animal Production and Health Division, Food and Agriculture Organization, Rome, Italy

Member governments of the United Nations' Food and Agriculture Organization (FAO) have resolved to have FAO design, coordinate, and facilitate the implementation and maintenance of a global program for the management of animal genetic resources. This program is aimed at 1) documenting, monitoring, and better understanding the breeds of livestock developed for food and other agricultural needs, 2) better developing and using this genetic material to achieve highly productive and sustainable agriculture throughout the world; and 3) maintaining for possible future use those unique genetic resources of little current interest to farming communities. The program is ready for launch and comprises 1) a country-driven global structure; 2) an intergovernmental mechanism to enable governments to monitor progress and to develop international policy, particularly for access to and maintenance of genetic resources; 3) a technical work program; and 4) a cadre of experts to help guide the program's development over the coming decades. Success will require the active involvement of governments and nongovernmental organizations, including private sector companies, research and training institutions, and international agencies. Each country is being invited to identify a national focal point and country contact for the program and to facilitate and to coordinate a within-country network. The program must also harmonize with the Convention on Biological Diversity to maximize opportunities for success and to minimize duplication.

5 Animal germplasm programs in North America

R.J. GERRITS, National Program Staff, USDA-ARS, Beltsville, MD, and **J.N.B. Shrestha**, Centre for Food and Animal Research, Agriculture and Agri-Food Canada, Ottawa, ON, Canada

The importance of biological diversity in all life forms, including animals, has received international attention in recent years. In 1990, national programs to protect animal genetic resources were initiated in the United States and Canada. The Centre for Food and Animal Research (CFAR) received funding to provide the leadership for the conservation of animal genetic resources in Canada. The Canadian program was developed as a working partnership among CFAR and other interested animal industry groups. Their objectives were to establish program guidelines, an inventory system, a gene bank for preservation, and research on the conservation of animal genetic resources. The U.S. National Animal Germplasm Program (NAGP) was established in the 1990 Farm Bill. The program is administered by the Secretary of the USDA through the ARS. The NAGP was established by a national committee comprised of Federal, university, and industry scientists. The objectives of the NAGP are to characterize, to utilize, and to preserve animal germplasm and to map and to identify genes associated with desirable production traits and disease resistance. Both countries have established repositories and germplasm database systems to track and preserve unique, diverse, and valuable animal germplasm.

6 Development of detailed microsatellite maps in livestock

C.W. BEATTIE, U.S. Meat Animal Research Center, USDA-ARS, Clay Center, NE

Remarkable progress has recently been made in the development of low resolution genetic linkage maps for the major livestock species. However, the several linkage maps now available for domestic species are not integrated, and estimates of overall coverage (genome size) are not robust. Continued development of high resolution linkage maps will require constructing integrated framework as well as comprehensive maps, which in turn, requires increasing marker density in areas of low resolution. A strategy of directed (linkage) mapping that incorporates microdissection and microcloning of specific chromosomal regions, direct selection of large genomic DNA inserts containing polymorphic markers, and direct chromosomal amplification of previously linked loci will increase marker density in specific regions. This strategy will also aid integration of the genetic and physical maps of individual species by providing framework markers. When this strategy is extended to closely related species (e.g., cattle and sheep), well ordered comparative linkage maps can be developed that allow marker integration across species lines.

7 Chromosome-specific libraries and use in swine genome mapping

J.K. LUNNEY, **D.R. Grimm**, **R. Holley-Shanks**, and **T. Goldman**, Parasite Immunobiology Laboratory, USDA-ARS, Beltsville, MD; **A. Ponce De Leon** and **S. Ambady**, Department of Veterinary and Animal Sciences, University of Massachusetts, Amherst, MA; and **J. Mendiola**, **L. Buoen**, and **C. Louis**, Department of Veterinary PathoBiology, University of Minnesota, St. Paul, MN

Swine chromosome-specific libraries have been prepared using flow cytometry and microdissection. The target has been to produce more swine chromosome-6 markers so that unique loci that determine favorable swine carcass traits known to map near the porcine stress syndrome defect on chromosome 6 can be identified. For flow cytometry, swine chromosomes were prepared from mitogen-activated lymphoblasts using polyamine buffers; these chromosomes were sorted based on their staining with Hoechst 33258 and chromomycin A3. The PCR-amplified, size-selected DNA from 100 sorted chromosomes was used for pBluescript and λ-ZAP libraries. As an alternate approach, 10 copies of chromosome 6 were isolated from chromosome spreads using microdissection, PCR amplified using microdrops and specifically designed primers, and then ligated into pBluescript. Purity of chromosome-enriched populations was verified by FISH analyses. Libraries were screened with di- and trinucleotide repeat probes and, after sequencing, PCR primers designed for each marker. To date, two chromosome-6 microsatellite markers have been identified and mapped; another 30 markers have been sequenced.

8 Mapping and the chicken: expressed sequence tags and genetic regulatory elements

S.J. LAMONT, Department of Animal Science, Iowa State University, Ames, IA

Mapping the chicken genome is rapidly progressing by using a variety of mapping technologies. These technologies include RFLP analysis with candidate gene probes, expressed sequence tags (EST) and random cloned DNA, blood group typing, and PCR analysis with RAPD markers, CR1 repeat elements, and microsatellite markers. Our emphasis on avian immunogenetics has led to focusing recent mapping efforts in two areas. The first is RFLP mapping of EST from a T-cell derived library through a collaborative project aimed at contributing anchor loci to build a consensus map from two international chicken genome maps (Compton, United Kingdom, and East Lansing, MI). Loci detected by cDNA probes from the Compton laboratory were RFLP-mapped in the East Lansing mapping reference population. The second research area is fine mapping the regulatory elements of the major histocompatibility complex (MHC) class II genes by sequence homology with known structural elements and subsequent functional characterization. Transfection studies have provided functional confirmation of both positive and negative regulatory elements in the 5′ flanking region of chicken MHC class II genes and the ability to modulate these regulatory elements by exogenous factors.

9 The map and its use in sheep

N.E. MUGGLI-COCKETT, Department of Animal, Dairy and Veterinary Sciences, Utah State University, Logan, UT

Genome scanning is a method in which many genetic markers spread across the genome are tested for genetic linkage with traits of interest. Many researchers are using genome scanning to identify economic trait loci (ETL) of livestock. An advantage of this method is that no prior knowledge of biological pathways or similar traits is needed. By including enough genetic markers into the analysis, all chromosomes can be systematically examined for association. Approximately 200 equally spaced markers will be necessary to scan the genome of livestock species completely. Using the genome scanning approach, markers have been identified that are linked to two ETL of sheep; these traits include the Booroola fecundity gene (Fec^B) responsible for high ovulation rate and the callipyge gene (CLPG) associated with muscle hypertrophy in the hind quarters. In both studies, once the first marker was linked to the trait, additional markers previously mapped to the corresponding regions of sheep, cattle, and humans were tested for association. In this way, the chromosomal areas around the ETL were finely mapped. Although genetic markers associated with the traits have been identified in these studies, the causative genes are not known at this time. Other methods such as chromosome walking and representational difference analysis will be needed to isolate the genes.

10 Integrating the syntenic, physical, and linkage maps

J.E. WOMACK, Department of Veterinary Pathobiology, Texas A&M University, College Station, TX

Multiple mapping methods have been applied to animal genomes, each resulting in a unique map with its own intrinsic value. Integration of maps is important because it enhances the value of the sum of genomic information and also provides an internal test for accuracy and reliability of this information. Integration of maps simply requires mapping the same locus by multiple methods. In practice, however, this is not always a simple task. Type I markers, because of their value in comparative mapping, are most often used for synteny and cytogenetic mapping. Type II markers, by definition highly polymorphic, are more likely to comprise the backbone of linkage maps. Integration of maps has been accomplished by incorporating Type I markers into linkage maps. This is an extremely important exercise for comparative mapping and the eventual extrapolation of data from the map-rich genomes of mouse and man to the map-poor genomes of livestock species. New techniques are available to identify polymorphisms of Type I markers, and interspecific backcrosses can be used to enhance allelic segregation. Linkage maps have been integrated with cytogenetic maps through the in situ hybridization of cosmids containing microsatellites. Recent success with in situ PCR suggests that integration can also be accomplished by direct cytogenetic mapping of the microsatellites that comprise the linkage map.

11 Mapping genes for disease resistance: lessons from the bovine leukemia virus

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Despite dramatic improvements in management practices, infectious diseases are still a major liability to producers of food animals. The development of low resolution linkage maps of farm animal genomes allows for new opportunities to map genes that affect disease resistance and susceptibility. A proven strategy for mapping loci that affect host resistance to infectious diseases (IDL) is classical linkage analysis. For mapping IDL by linkage analysis, a suitable population of animals scored for a disease phenotype(s) is needed. Disease traits can be categorical (e.g., with or without mastitis) or have a continuous distribution (e.g., somatic cell count). Mapping IDL can be achieved by genome scanning or the candidate gene approach, which may utilize comparative mapping information (e.g., the major histocompatibility complex and enzootic bovine leukosis). New technologies with the potential for cloning of segments that are tightly linked to IDL include genome mismatch scanning and representational difference analysis. These new methods could eliminate the need for scoring large numbers of markers, thus conserving time and effort. These strategies are, at least in theory, applicable to outbred populations and family structures that are presently being used to map economic trait loci of dairy and beef cattle.

12 In vitro maturation and fertilization of pig oocytes

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Recent research progress on in vitro maturation (IVM) and fertilization (IVF) of porcine oocytes has resulted in an increase in the incidence of male pronuclear formation and has demonstrated detrimental effects of elevated NaCl concentration in the maturation medium as well as the importance of oocyte glutathione content for cytoplasmic maturation of oocytes. In addition, pretreatment of spermatozoa and enhancement of culture conditions during IVM has been determined to increase the incidence of monospermic penetration. However, a relatively high rate of polyspermic penetration, which could be derived from an unstable cortical reaction, and a large variation in embryonic developmental rate to day 30 of pregnancy occurs even if the oocytes are matured and fertilized by use of the most recent technological advancements. Traditional systems for IVM are considerably different from the final follicular environment prior to the luteinizing hormone surge. Culture conditions of oocyte-cumulus complexes prior to exposure to gonadotropins appear to have important effects on the developmental ability following IVM and IVF. Future research interests are to clarify the mechanism involved in the establishment of developmental competence in oocytes during the final phases of folliculogenesis and to achieve a higher incidence of monospermic penetration following IVF.

13 Factors influencing development of embryos produced by nuclear transfer

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Many factors influence development of embryos produced by nuclear transfer, including species, donor embryo stage, choice of recipient cytoplast, method of activation of the oocyte, and culture system for the embryo. Another factor that has been shown to have great impact is the coordination of cycles of donor and recipient cells. If a nucleus is transferred into an oocyte at metaphase II, then DNA replication is induced regardless of the stage of the donor cell cycle. Hence, it is essential that donor cells are in G1 phase of the cell cycle. By contrast, if the oocyte is activated and cultured to allow the decay of maturation promoting factor activity, then the nucleus determines whether or not replication should occur. Such a cytoplast is suitable for nuclei at any stage of the cell cycle. By use of a "universal recipient," the proportion of embryos that develop has been increased, and development to term has been obtained with nuclei from later stages of development, including cultured embryonic cells. Nuclear transfer from cultured embryonic cells would permit dissemination of genetic improvement by embryo multiplication and the introduction of precise genetic changes by gene targeting.

14 Gender preselection in mammals

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Recent advances in separation of X and Y chromosome-bearing spermatozoa have led to the availability of a method (Beltsville Sperm Sexing Technology) to preselect the sex of several mammals: cattle, sheep, swine, and rabbits. Individual sperm DNA can be determined and used as the differentiating characteristic with flow cytometry and cell-sorting instrumentation especially modified to measure small amounts of DNA in sperm. The process utilizes the fluorochrome Hoechst 33342 to bind to the DNA. The sorted sperm are used to inseminate eggs via in vitro fertilization (IVF) or by surgical insemination into the oviduct or uterus of appropriate females. Sperm are sorted at .3 to .5 million per hour for most species with the expectation that progeny will be ≥85% X or Y. Using IVF, cattle progeny have been produced at ≥90% accuracy. Sexed progeny of rabbits and pigs have also been produced with similar accuracy. All progeny produced (≥300) have exhibited completely normal morphological appearance and normal reproductive function. The technology has also been applied to human sperm. Clinical tests with human sperm by the Genetics and IVF Institute, Fairfax, VA, are currently in progress. This sexing technology is applicable where IVF, intrauterine, or intratubal insemination are convenient means for producing offspring.

15 Modification of milk composition in transgenic animals

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Amidst the explosion of fundamental knowledge generated from transgenic animal models, a small group of scientists have been producing transgenic livestock to test the feasibility of improving animal production efficiency and generating new products. Modifying the genetic control of mammary glands provides an opportunity to pursue several distinctly different goals. The objective of the emerging "gene pharming" industry is to produce drugs for treating human diseases. It is argued that mammary glands are an ideal site for producing complex bioactive proteins that can be harvested and purified in a cost effective manner. Several products produced in this way are approaching clinical trials. A second strategy, which has been widely discussed but has received less attention in the laboratory, focuses on altering the ratio of endogenous milk proteins to improve the nutritional quality of milk and to reduce processing costs of dairy products. Proposals include increasing protein, decreasing fat, altering physical properties, and "humanizing" milk. The potential profitability of gene pharming seems clear; however, cost-benefits of genetically engineered dairy products will remain marginal until inefficiencies of transgenic technology such as low gene integration rates, poor embryo survival, and unpredictable transgene behavior are overcome.

16 Embryonic stem cells for farm animals

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Domestic animal embryonic stem (ES) cell technology will have a major impact on future attempts to modify and to improve agricultural animals genetically. This field has made some major strides in recent years. Pluripotent stem cell lines or ES-like cells have been propagated in vitro while maintaining pluripotent properties. Domestic animal ES-like cells allowed to differentiate in vitro formed cell types representative of all three germ layers. Cell types ranged from ciliated epithelium to multinucleated striated muscle. More recently, ES-like cells have contributed to developing fetuses and offspring. Nuclear transfer studies using ES-like cells as donor nuclei have tested the in vivo developmental capacity of cultured embryonic cells. Donor nuclei from established bovine ES-like cell lines directed fetal development through organogenesis but lacked the ability to direct normal placental development. However, cultured bovine and ovine inner cell mass cells have given rise to nuclear transfer offspring. Phenotypic evidence of porcine ES-like cell line contribution to chimeric offspring has also been observed. In the end, for ES-like cell lines to be of value, germline transmission must be demonstrated. Once accomplished, germline transmittable farm animal ES cells will be a tremendous asset to cloning and transgenic animal technologies.

17 Primordial germ cells for genetic modification of poultry

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Research has been directed toward sterile hosts, long-term culture of chick embryonic stem (ES) cells, transfection of turkey gonadal primordial germ cells (gPGC), transfer of transfected gPGC to host turkey embroys (F_0), and recovery of DNA transgenes from F_0 and F_1 turkeys. The injection of embryonated chicken eggs with EMA-1 antibody and the immunization of hens with germ cell-like 500 Kd antigen resulted in sterile host embryos. Stage-X blastodermal cells have been subcultured for 25 generations. These ES cells DiI transfer to gonads. The transfection efficiency of primary cultured gPGC exposed to either lacZ or antisense prolaction (asPRL) DNA using Lipofectomine® as the carrier was 26%. These gPGC were transferred to stage-17 host embryos. The exogenous DNA was detected in the turkey F_0 embryonic gonad F_1 muscle by Slot Blot with a ^{32}P probe for lacZ or by Southern Blot with a ^{32}P asPRL probe. Gametes containing the foreign DNA were recovered from F_0 and F_1 turkeys. This nonviral method of gene transfer provides germline modification (transgenic birds).

18 Food and Drug Administration concerns regarding genetic improvement of farm animals

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The biotechnology explosion that began in the 1970's has led to the development of techniques enabling molecular biologists to manipulate the germline of a variety of animals directly through the introduction of new genetic material in the form of DNA fragments. The newly introduced DNA becomes part of the genome of the animal and can be inherited by subsequent generations. The techniques of transgenesis allow both removal of undesired traits and introduction of new traits. An enormous number of applications of this technique can be envisioned and are being developed. These applications include use of transgenic animals as producers of biological therapeutics or as human disease models for testing of potential pharmaceutical agents. Depending on the application, several Federal agencies can be involved in regulation of the use of transgenic animals. The Food and Drug Administration (FDA) regulates the use of transgenic animals through scientific assessment of the risks and benefits associated with the use of this technique. Issues beyond the scope of this scientific risk/benefit assessment are considered in conjunction with outside advisory bodies, including the National Institute of Health Recombinant DNA Advisory Committee and FDA advisory committees convened to give advice on specific issues arising from the use of biotechnology.

19 Acceptance of biotechnology: Food Safety and Inspection Service concerns

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Recent progress in the field of biotechnology and the production of transgenic livestock has raised the need for guidance on the disposition of these animals at slaughter. National surveys in the United States and in Great Britain have shown that, although the public knowledge of biotechnology has increased, the use of this technology is more acceptable in human medicine and in production of plants than in the production of transgenic food animals. Therefore, there is a need to regulate food animals resulting from transgenic animal research. Several governmental agencies are anticipated to be involved in regulatory issues pertaining to these animals. The Food Safety and Inspection Service (FSIS) of USDA will ultimately be responsible for ensuring that transgenic animals intended for human consumption are wholesome, unadulterated, and properly labeled. Scientists in FSIS, in conjunction with other governmental agencies and the Agricultural Biotechnology Research Advisory Committee, have developed guidelines for the slaughter of animals resulting from transgenic research.

20 Public perceptions of animal biotechnology

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Biotechnology has evolved from the genetic manipulation of microbes to the development of transgenic animals. Although the public tacitly accepted chymosin produced by genetically altered microbes for use in the manufacture of cheese, the use of animals in genetic engineering engenders intense public interest and opposition. International studies show that the world public is more opposed to genetic engineering in animals than applications to plants or microbes. Although the potential uses of genetic engineering in animals are significant for the global food supply, public acceptance is mandatory for continued advancement. Efforts must be made to understand public perceptions of the issues unique to genetic engineering in animals as well as the general issues surrounding genetic engineering. Some of the special issues include ethical concern for preserving genetic integrity, concern for the well-being of animals as sentient beings, and potential impacts on genetic diversity. Animal genetic engineering must also deal with the more general lack of public understanding of biotechnology. By recognizing these public perception issues, industry, scientists, and regulators will be better able to assess acceptable uses of biotechnology, to consider ethical issues in designing projects, and to address public concerns early in the process.

21 Congressional concerns regarding biotechnology

W. Allard, Colorado 4th Congressional District, U.S. House of Representatives, Washington, DC

Abstract not available

22 Introduction to QTL detection and marker-assisted selection

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Most economically important traits of livestock are determined by the joint effects of many environmental and genetic factors. Traditional trait-based breeding methodologies utilize only a fraction of the genetic variation present in outbred populations and are limited by expression of most traits only for females. Marker-assisted selection (MAS) should make possible an increase in the accuracy of genetic evaluations, an increase in selection intensity, a decrease in generation interval, and better utilization of nonadditive genetic variation. The advantage of MAS will be greatest for situations in which traditional index selection is inefficient because of long generation interval, low heritability, or index selection for negatively correlated traits. Individual loci that affect QTL can be detected with the aid of genetic markers via genetic linkage. For outbred populations, QTL have been detected by analysis of large half-sib or full-sib families. The power of detection per genotype can be increased by selective genotyping, replicate progeny, and pooling of genotypes. New statistical methods to combine marker data for several traits of many individuals are required.

23 Mapping genes that influence meat characteristics of pigs

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The development of powerful tools for genome analysis opens up possibilities for genetic dissection of complex phenotypic traits. Current state-of-the-art mapping and identification of genes controlling meat traits have been reported for pigs. Two major genes affecting meat characteristics have been identified: halothane (HAL) and rendement napole (RN). The HAL-associated phenotype results from a point mutation in the calcium release channel. The gene for the RN mutation has not yet been found, but mapping is progressing. Crosses between divergent populations have recently been utilized for the identification of major genes for fatness traits. Use of complex segregation analyses for the identification of a major gene for high intramuscular fat present in a Meishan × Large White cross have been reported, and mapping the gene using available pedigree information should be straightforward. A cross between wild and Large White do-mestic pigs has been used to identify a QTL on chromosome 4 with a major effect on fatness.

24 Improving dairy cattle using marker data

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Use of molecular data will require dense genomic maps of the bovine with sufficiently polymorphic markers to track QTL. Use of these maps for dairy cattle improvement is facilitated by multigenerational pedigree structures and organized performance testing. Once QTL-marker associations are accurately estimated, young animals with favorable allelic combinations can be selected. Continued performance recording and evaluation of marker-QTL associations will be required for several reasons. Initial associations of markers and QTL will be family specific and will need estimation in each generation to detect recombination. Even extremely close markers that do not recombine with QTL will acquire other positive or negative linkages because of recombinations away from initial marker-QTL position. Secondly, as some QTL alleles reach high frequencies, marker-assisted selection will shift to other loci not yet utilized. Thirdly, traits under selection are likely to change so that the new selection goals or traits will require estimates of desirable marker-QTL combinations with different economic weighting. In each case, marker data will be combined with performance data for the individual and for relatives to make optimal progress. Marker-assisted selection will provide additional progress if marker information can be accurately and efficiently included in ongoing selection programs.

25 The use of markers in poultry improvement

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Markers have several uses in poultry improvement: 1) a tool for understanding the genome and how it works, 2) genomic selection, 3) establishment of pedigrees, 4) prediction of heterosis, and 5) marker-assisted selection. Expediting learning about the genome and how it works is the most important facet of marker technology because knowledge is the basis of authentic progress. For poultry, useful genes (such as genes to allow "autosexing" at hatch or genes to reduce heat stress) have been introduced into commercial and other important stocks. This required several generations of backcrossing to allow recombination and to permit the elimination of unwanted genes but with no assurance that all undesirable genes would be eliminated. Genomic selection using markers would greatly accelerate the introgression of useful genes. Flanking markers would ensure that only the desired gene was introduced. Markers may provide a useful basis for predicting heterosis and other genetic effects associated with crossing. Commercial performance is enhanced by taking advantage of heterosis and other crossing effects in many stocks of poultry. Markers provide the possibility of determining pedigrees or assessing inbreeding levels in stocks for which that information may not be obtainable otherwise. Finally, marker-assisted selection may allow opportunities for enhanced improvement, especially in cases where direct selection would not otherwise be possible.

26 Databases and information systems needed for maps and marker-assisted selection

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Maintaining a current database and information system with marker sequences and genotypic data is critical to development and utilization of physically anchored genetic linkage maps. Building the most accurate consensus genetic linkage map possible requires simultaneous analysis of genotypic data from all sources. High resolution physical mapping and positional cloning is not highly effective until resolution of the genetic linkage map reaches 2 cM. Ordering markers spaced 2 cM apart requires at least 220 coinformative meioses, which in turn requires a large number animals because of incomplete marker informativeness. Acquiring genotypic data for large numbers of markers and animals is likely to require contributions from many laboratories. The integrity of genetic maps is highly sensitive to genotyping errors. Integrating genotypic data from different sources increases the probability of finding and correcting genotyping errors. Standardized marker names and allele sizes are required for data integration. Marker names are usually based on function or chromosomal location. Names change as knowledge changes. Use of arbitrary marker names helps but does not solve the problem because the same marker can be developed independently by different investigators. Sequence analysis can be effectively used to identify duplicate markers.

27 Integrating use of new markers into the real world

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In theory, all animal breeders could apply genetic marker technology and marker-assisted selection to speed improvement of their populations. In reality, the income from each new biotechnology must exceed its cost before it becomes useful. Fairly small advantages from genotyping will be profitable if reproductive rates are high. Genotyping of most females and of embryos will not be profitable given current reproductive rates. Success of marker-assisted selection depends greatly on the presence of a few genes with moderate or large effect. To estimate allele differences, breeders will genotype siblings in each elite family that have phenotype or progeny-test data. If allele differences are large at a particular locus, breeders will also genotype younger family members without data to shortcut the selection process. Most livestock families are too small to allow precise estimation of quantitative gene parameters regardless of how many markers are tested. Estimated effects should be regressed for expected error content before use in breeding values. Industry cooperation will be needed to collect, to interpret, and to report genotypic data, especially if family members are widely dispersed and if different laboratories use different markers. Genetic markers are a sensible investment, especially considering that genetic selection has improved animal performance much more than all the other sciences.

POSTER ABSTRACTS

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P1 Characterization of equine major histocompatibility complex loci DRA and DRB using single-strand conformation polymorphism

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Single-strand conformation polymorphism (SSCP) gel electrophoresis was used to identify three alleles of the equine DRA locus previously characterized by allele-specific PCR and sequencing. Furthermore, additional SSCP bands were identified among the other equid species and include one new allele for kulans. The second exon of equine DRB was characterized similarly. The SSCP gels presented a more complex pattern, with phenotypes exhibiting between 4 and 13 bands. The DRB SSCP patterns were studied for two families (6 to 13 bands per pattern). For both families, the patterns showed simple Mendelian inheritance. For individuals of one family, the PCR products were cloned and retested on SSCP gels. All the bands derived from genomic DNA amplification could be accounted for with bands derived from the cloned DNA amplification products. The results are consistent with those for horses with three DRB loci. Individuals with as few as 4 SSCP bands for DRB may possess only two DRB loci. These studies do not indicate whether or not these loci are expressed.

P2 Efforts towards mapping the shrimp genome

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Performance records for penaeid shrimp are being generated through a selective breeding program using four specific pathogen-free (SPF) or candidate SPF *Penaeus vannamei* populations developed by the U.S. Marine Shrimp Farming Program. The breeding program relies not only on heritability estimates for size and disease resistance but also on molecular genetic approaches. Research goals are 1) to monitor genetic diversity of SPF shrimp using molecular genetic techniques, 2) to follow stock identification using mitochondrial DNA (mtDNA) RFLP, 3) to identify population-specific markers using RAPD, and 4) to use microsatellites for mapping loci for growth and disease resistance and susceptibility. Data from 3 years indicate that 1) nuclear and mtDNA polymorphisms are more useful than allozyme polymorphisms for examining genetic diversity, 2) variable levels of genetic diversity (45 to 76%) are found in SPF populations, 3) mtDNA RFLP can be used for stock identification, and 4) a unique population-specific RAPD marker (B-20) has been found. Sequencing revealed two microsatellites in B-20, which makes them ideal markers to begin efforts towards mapping the *P. vannamei* shrimp genome.

P3 The use of transgenes to alter the physical and functional properties of milk

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The development of transgenic technology holds great promise for the eventual direct manipulation of the genomes of domestic animals for the purpose of altering economically important production traits. A long-term project was undertaken to alter the protein composition (or functional nature) of proteins that occur in milk. Transgenic lines of mice have been produced that express either the human lysozyme protein or a modified bovine κ-casein protein under the control of mammary gland-specific promoters. The transgene constructs expressed at the messenger RNA and protein levels in a manner appropriate for development stage and tissue specificity. The expression of these proteins in milk resulted in decreased rennet clotting time and increased curd strength (lysozyme) or decreased micelle size and increased curd strength (κ-casein). Although transgenic mouse milk containing human lysozyme had smaller (but not significantly different) micelles when compared with control milk, the presence of the modified bovine κ-casein in mouse milk had no effect on rennet clotting time. These results demonstrate the feasibility of using transgenic technology to alter the functional properties of the milk protein system and suggest that appropriate modifications for dairy cattle could result in altered manufacturing properties for the milk.

P4 Construction and analysis of a heterogeneous nuclear cDNA library of the p-arm of pig chromosome 12

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To isolate unknown genes on pig chromosomes, a method was used that relies on the presence of pig-specific repetitive sequences in heterogeneous nuclear cDNA (hncDNA). Heterogenneous nuclear RNA was prepared from hamster × pig somatic cell hybrid 8990, which contains only the p-arm of pig chromosome 12 in a hamster background. To obtain hncDNA from this RNA, first-strand synthesis was carried out using a random primer. This was followed by specific amplification of pig sequences using a combination of two pig SINE primers. Products were size selected on agarose, extracted, and cloned into the vector PCR-Script. The library is being characterized in terms of the number of clones obtained with inserts, the pig specificity of insert sequences, and the number of these that have open reading frames.

P5 Parentage determination for sheep using microsatellites

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The high polymorphism exhibited by short tandem repeat loci make them appropriate for genetic studies, particularly for parentage testing. Efficiency of some microsatellites in determination of parentage was evaluated for sheep. The animals examined belong to the Spanish Churra breed and are included in a selection program. The study included 172 ewe-lamb pairs from 11 sires (4 natural service and 7 AI). Five microsatellites were genotyped on DNA obtained either from blood or frozen semen. The markers studied and the number of alleles detected in each locus were MAF4 (18), MAF64 (12), OarAE101 (9), OarJMP8 (7), and BM4621 (13). Values of the polymorphism information content estimated in these loci were .913, .721, .673, .684 and .839, respectively. The microsatellite with the largest exclusion probability was MAF4; the overall value obtained for the five loci was 99.5%. For cases in which paternity incompatibility was detected, an attempt was made to obtain the correct assignment.

P6 Identification of somatic cell score QTL of Holstein cattle

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Many genes, as well as environmental effects, may affect differences in a measurable phenotype such as mastitis susceptibility. Possible QTL for mastitis were investigated using somatic cell score (SCS) as the mastitis trait. Semen DNA samples from the Dairy Bull DNA Repository were genotyped using microsatellites from the Meat Animal Research Center map. Seven grandsires with ≥50 sons with reliabilities >50% were selected. Sons with the 10 highest and 10 lowest predicted transmitting abilities (PTA) for SCS were genotyped with 19 microsatellite markers from 7 chromosomes using granddaughter records as trait endpoints. Data were analyzed using genotype within family as the independent variable and PTA for SCS as the dependent variable. Three markers (two on chromosome 23 and one on chromosome 17) showed significance in one of four families. One marker on chromosome 23 showed significance in three of five polymorphic families. Additional sons from these families will be evaluated to determine the unbiased effect across and within families for these markers. These results demonstrate that QTL within and possibly across families can be mapped with microsatellite markers in a granddaughter design.

P7 American breeds as genetic resources

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A livestock breed is a defined genetic package with a predictable range of characteristics. Farm animal breeds have both scientific and economic importance because they have undergone complex adaptations to a variety of climates, forages, parasites and diseases, and management systems while maintaining health and vigor. In the quest to improve farm animals, attention must be given to conservation of breeds that have the potential to contribute to a variety of animal production systems. Many genetically important breeds are now rare, and these rare breeds are the focus of conservation and research programs by the American Livestock Breeds Conservancy. American breeds of both genetic significance and immediate commercial value include Myotonic and Spanish meat goats, Gulf Coast Native sheep, Caribbean Hair sheep, Florida Cracker and Pineywoods cattle, Dutch Belted cattle, Bronze turkeys, and Tamworth swine. The American Livestock Breeds Conservancy, a nonprofit organization, is part of the global animal genetic resources network.

P8 Improved wool production from transgenic sheep expressing insulin-like growth factor I driven by a keratin promoter

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An ultra-high sulfur keratin (KER) promoter was used to target expression of chloramphenicol acetyl transferase (CAT) to the wool follicle of transgenic sheep. Four of 31 lambs were transgenic, and one ram showed CAT expression specifically in the keratogenous zone of the wool follicle. When wool production was manipulated by altering the nutrition plane, CAT expression was related linearly to rate of wool growth. The KER promoter was linked to a sheep insulin-like growth factor I (IGF1) cDNA, and five transgenic lambs were produced; one male and one female showed IGF1 expression in the skin. A progeny test of the ram was carried out by matings to 43 nontransgenic ewes. Of 85 lambs born, 43 (50.6%) were transgenic. At hogget shearing (approximately 14 months of age), clean fleece weight (grams per kilogram of body weight) was on average 10% greater in transgenics than in their nontransgenic half-sibs. Rate of wool growth was $10.5 \pm .2$ g/day (mean ± SD) for transgenics compared with $9.7 \pm .2$ g/day for nontransgenics. There were no significant differences between the two groups in hogget body weight, rate of body weight gain, or mean fiber diameter. Data for other fiber characteristics, including length, strength, medullation, and bulk, are currently being analyzed.

P9 Quantification of bovine cytokine gene expression with the ribonuclease protection assay and competitive RT-PCR

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To assess the levels of cytokine gene expression in cattle and to correlate those levels to host resistance to gastrointestinal nematodes, two assays to quantify messenger RNA expression for bovine cytokines involved in T-cell growth and development were devised. Specific probes for bovine interleukin 2 (IL-2), IL-4, IL-10, IL-12, and γ-interferon were developed and used in ribonuclese protection assays in stimulated peripheral blood mononuclear cell and lymph node cell populations. For lower levels of cytokine expression in nonstimulated populations, quantitative RT-PCR was also developed. A novel technique for the generation of competitor molecules was devised. This technique, which requires a single PCR amplification, uses internal primers separated by a predefined distance that amplify in opposite directions to encompass the vector. As a result, the molecules amplified from the competitor DNA are smaller than the naturally derived cDNA sequence and do not require further restriction enzyme digestion. This method allowed the quantification of bovine IL-10 gene expression from stimulated and nonstimulated populations without the need for radioisotope labeling. These assays will allow the delineation of the mechanisms by which cattle become immune to these economically important parasites.

P10 Major histocompatibility complex genes as possible selection criteria to improve reproduction and wholesomeness of sheep

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The major histocompatibility complex (MHC) can influence reproductive traits as well as resistance and susceptibility to diseases. Research objectives were to analyze 1) the relationship between MHC class I and reproductive performance (frequency and intensity of fetomaternal immunizations and their effect on ewe fertility and lamb rearing) and 2) the influence of the MHC class II DRB gene on sheep resistance to mastitis. Results of this investigation should allow proper selection of markers to improve reproduction and wholesomeness of sheep. Fetomaternal immunizations were determined based on lymphocytotoxic effect of dam blood sera on lymphocytes of offspring of 144 Polish Merino ewes. Occurrence of mastitis was determined by somatic cell count and presence of bacteria in milk of 105 Polish Lowland sheep examined three times during lactation. Molecular analysis was carried out, and alleles were recorded for 1) MHC class I region, domain α3, exon 4 (RFLP) and 2) a microsatellite in the MHC class II DRB gene closely linked to exon 2. Analysis showed a distinct polymorphism in the microsatellite length and a less conspicuous polymorphism for the RFLP in the MHC class I region. Differences in the frequency of polymorphic MHC class I and II fragments associated with reproductive traits and health of sheep were determined.

P11 Identification and synteny mapping of equine microsatellite DNA markers

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Construction of the equine gene map lags behind progress made for other domestic animals. Nevertheless, a gene map will be a valuable tool for horse breeders. A panel of mouse-horse heterohybridoma cells has been constructed and is being used to identify synteny groups between microsatellite markers and anchor loci. Using PCR, DNA isolated from the heterohybridoma clones was screened for the presence of different markers, which included published microsatellite markers, microsatellite markers developed at the University of Kentucky, and PCR detection of known coding genes such as major histocompatibility complex DRA and transferrin (anchor loci). To date, 35 markers have been tested and 6 synteny groups that include 20 of these markers have been identified. The other 15 markers exhibit distinct reaction patterns on the panel. The resulting synteny map will be a valuable guide for selection of markers for use in creating a linkage map.

P12 Genetic polymorphism at the κ-casein and β-lactoglobulin loci in Brazilian cattle and buffalo populations

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Genetic polymorphism at the κ-casein and β-lactoglobulin loci was investigated in blood samples from two Brazilian *Bos indicus* breeds (127 Nellore and Gyr animals) and from 15 Brazilian river water buffalo. Genetic variants A and B were identified in the two loci by using PCR techniques. The amplification of buffalo DNA with primers designed for cattle DNA and the similar size of the amplified segments indicated that this region is conserved in both genera. No genetic variation was detected in the buffalo population, which exhibited κ-casein B and β-lactoglobulin B alleles. Respective frequencies of B allele in the κ-casein and β-lactoglobulin loci were .08 and .77 in Nellore and .06 and .62 in Gyr. Allele frequencies of κ-casein B for Brazilian populations were lower than those reported for original Indian breeds; no significant differences were observed between populations for the β-lactoglobulin locus. Lower frequencies of κ-casein B allele could be achieved by appropriate single gene selection strategies, thus improving the manufacturing properties of the milk supply in Brazil.

P13 Germplasm characterization and preservation for Colombian Criollo cattle

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The history of cattle in Colombia dates from the 1530's when animals arrived from Hispaniola in sufficient numbers to establish breeding herds around the present city of Bogota. Today, their descendants are recognized as individual Criollo breeds —each with their own unique genetic attributes. In collaboration with federal and private Colombian agencies, a project has been initiated to characterize some of these unique cattle breeds genetically. Specific focus is on germplasm characterization and preservation for the seven most common and economically important breeds of Colombian Criollo cattle (Blanco Orejinegro, Casanare, Chino Santandereano, Costeno con Cuernos, Harton del Valle, Romosinuano, and San Martinero). Based on nucleotide sequence information from the mitochondrial genome and hypervariable microsatellite markers from the nuclear genome, objectives are 1) to quantify genetic uniqueness of each breed, 2) to determine phylogenetic relationships and origin of each breed, and 3) to map genetic characters of economic importance unique to each of these breeds with microsatellite markers. Targeted genetic traits include disease resistance and tolerance, production traits (meat and milk), behavior traits, ability to thrive on poor quality roughage, and heat and altitude tolerance.

P14 Use of molecular genetic techniques to evaluate effects of environmental toxicants on genetic diversity of common loons (*Gavia immer*)

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Loss of biodiversity due to habitat destruction and environmental pollution are of major concern today. Correlation between genetic diversity and environmental toxicant burden was examined for common loons (*Gavia immer*). Genetic diversity was evaluated with RAPD and RFLP of mitochondrial cytochrome b (cyt b) gene. Thirty New Hampshire (NH) and Massachusetts (MA) birds and 14 Michigan (MI) birds were tested using 12 RAPD primers, and 178 RAPD bands were scored. Data indicated that NH and MA populations have more polymorphic bands than the MI population. A 307-bp fragment of loon cyt b was PCR-amplified, cloned, and sequenced. An RFLP analysis using BamHI, BsaJI, DdeI, DpnI, HaeIII, MseI, NlaIII and RsaI enzymes and ^{32}P-labeled cyt b probe showed distinct polymorphisms with BamHI enzyme only. Four cyt b haplotypes were detected in the MA population, three haplotypes in the NH population, and two haplotypes in the MI population. Preliminary analysis of mercury levels in a subsample of MA loons showed that birds with high mercury levels are less polymorphic than birds with low mercury levels.

P15 Expression of a murine hepatic lipase cDNA gene in porcine skeletal muscle

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The existence of gene expression was examined 10 days after direct transfer of DNA into pig gluteus medius (GM). The expression vector was pcDNA3 (invitrogen, 5.4 kb) harboring a 1.64-kb rat hepatic lipase (RHL) cDNA gene. India ink (100 µl) was added to 500 µl of PBS containing 300 µg of the vector. Three littermate pigs (7 days old) were injected in the right GM using a 1-cc syringe. The left GM of each pig was not injected (control). A blood sample was collected from each pig after gene transfer. After 10 days, the pigs were slaughtered and a 4-cm square GM sample was recovered from the injection area as marked by the India ink. Treated and control samles were frozen immediately. Blood samples were also collected at slaughter. Total RNA was prepared for a Northern blot. A .5-kb biotin-labeled RHL probe was used for analysis. Treated GM expressed the RHL gene, whereas control GM did not. Work is in progress to determine if tissue expression of RHL affected various blood components (cholesterol and high density lipoproteins).

P16 Alleles for the class II BoLA locus as risk factors for high somatic cell counts of lactating dairy cows

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Lactating Holsteins (1100 cows) from 93 dairy herds were genotyped for the major histocompatibility complex class II, BoLA DRB3.2 locus using a PCR-RFLP genotyping system. The DNA was obtained from milk samples after processing at a Dairy Herd Improvement Association (DHIA) facility. The DHIA somatic cell counts (SCC) were used to classify animals as 1) acutely elevated (one SCC test >500,000), 2) chronically elevated (three consecutive SCC tests >300,000 or two consecutive tests >500,000), or 3) eligible to be a control. For each acutely or chronically elevated cow, controls were selected based on breed, parity, herd, and lactation length (±60 days in milk). A conditional stepwise logistic regression model was used to determine the relative odds for the 10 alleles with a frequency greater than 5%. For 516 cows with elevated SCC and their controls, the class II BoLA DRB3.2*16 allele was significantly associated with the risk of acute increases in SCC. The DRB3.2*16 allele has been identified as a risk factor for acute intramammary infection, and the use of DHIA milk samples as a source of DNA useful for genetic epidemiologic studies has been established.

P17 Defined culture medium for porcine zygotes: Beltsville Embryo Culture Medium 3

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Media are available that can deliver modest embryonic development from a single-cell zygote to the blastocyst stage. However, few embryos develop to hatched blastocysts by day 7 in vitro, which indicates media deficiencies that inhibit early embryonic development. Beltsville Embryo Culture Medium 3 (BECM-3) was developed as a defined culture medium that can support development from single cell zygotes to the blastocyst stage. If 10% fetal bovine serum is added to BECM-3 at day 5 (late morulae/early blastocyst stage), over 90% of cleaved embryos cultured from day 1 develop into hatched blastocysts by day 7. Embryonic development from day 1 (1- and 2-cell embryos) to day-7 blastocysts in BECM-3 and NCSU-23 was compared: 100% (14 of 14) in BECM-3 and 62% (8 of 13) in NCSU-23. Hatched blastocyst development by day 7 was also compared: 93% (13 of 14) in BECM-3 and 0% (0 of 13) in NCSU-23. Mean cell number was threefold higher for embryos developing in BECM-3 (60.5) compared with those developing in NCSU-23 (21.2). These preliminary data show that BECM-3 can support normal morphological development to day-7 hatched blastocysts that have significantly increased cell numbers.

P18 An ovine growth hormone transgene model suitable for selection experiments with mice

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The oMT1a-oGH transgene of mice can be regulated by dietary zinc and provides a potential model for selection studies. To determine effect of oMT1a-oGH on growth and reproduction in the genetic background and environment in which selection would occur, males homozygous for the transgene were mated to females from high-growth (M16) and control (ICR) lines to create hemizygous transgenics, TM and TC, respectively. Progeny either did (Z) or did not receive (C) 25 mM $ZnSO_4$ from 3 (weaning) to 8 weeks. The TM mice had larger weekly body weights than TC mice from 3 to 10 weeks, and Z mice were larger than C mice from 5 to 10 weeks for TC mice and from 6 to 10 weeks for TM mice. Significant line-treatment interactions for weekly body weights from 4 to 8 weeks resulted from greater response to the transgene by TC mice than by TM mice. Compared with controls, male mice given Zn had reduced 8-week testis, epididymal, and subcutaneous fat pad weights as a percentage of body weight, but liver percentage was increased. The Z and C females did not differ in percentage of infertile matings and days from mating to littering, but Z females had larger litters. This transgenic model is suitable for selection studies; its expression increases growth rate, reduces fat content, and has no initial negative effect on fitness if regulated.

P19 Effect of exposure of porcine oocyte-cumulus complexes to gonadtropins

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Effect of delayed exposure of porcine oocyte-cumulus complexes (OCC) to gonadotropins on diameter of oocytes was examined along with the timing of germinal vesicle breakdown (GVBD) and the ability of inseminated oocytes to develop to the blastocyst stage following in vitro maturation (IVM) and fertilization (IVF). After preincubation (experimental) or no preincubation (control) in modified NCSU-23 medium for 12 hours, OCC were cultured in the same media supplemented with gonadotropins for 20 hours and then without those gonadotropins for 20 hours. The diameter of oocytes at the start of culture for maturation did not differ between the control and experimental groups. The incidence of GVBD after 20 hours of maturation culture was not different between the control and experimental groups. The incidence of embryos that developed in vitro to the blastocyst stage (23.1%) was higher for the experimental group as compared with the control group (8.7%). The blastocysts for the experimental group had a higher number of cells than control blastocysts. These results indicate that preincubation of porcine OCC before exposure to gonadotropic supplements for IVM increases the incidence of blastocyst formation following IVM and IVF.

P20 Genetically controlled resistance to gastrointestinal nematode infections of cattle

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A measure of parasite transmission for cattle is the number of parasite eggs in the feces (EPG). The heritability of EPG is .2 to .3, and repeatability is approximately .6. In addition, the risk that certain bulls will produce calves with diminished resistance (i.e., high EPG) is 25 times higher than that for other bulls. Using this information, Angus cattle are being selected for enhanced or diminished resistance to gastrointestinal nematodes in a herd homozygous for major histocompatibility complex loci. Resistance is determined by exposure to the parasites for a minimum of 120 days. To date, extensive immunologic and parasitologic data have been collected for 77 animals. Natural exposure to the parasites indicates that there are three phenotypes: 1) innate resistance, 2) acquired resistance, and 3) susceptibility. These phenotypes occur at a 1:2:1 ratio. However, if measured only at the end of the trial, there are 2 phenotypes: 1) resistant and 2) susceptible—at a 3:1 ratio. As the number of parasites in the host increases, there are 1) enlargement of the draining lymph nodes, 2) decreases in the percentage of $CD2^+$ cells, and 3) an increase in immunoglobulin-bearing cells. Preliminary results indicate that the decrease in T-cell percentages corresponds to decreased levels of interleukin 2 and γ-interferon mRNA in stimulated cells.

P21 Reorganization of genetic structures of cattle breeds as a result of selection influences

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Breed-specific traits and genetic structures differ among dairy, dual-purpose, and beef cattle. Associations between allelic frequencies for genetic-biochemical systems and productivity traits were discovered. Intrabreed genetic differences associated with specific local ecologic-geographic conditions were observed. These genetic differences indicated similarities within breed groups. Progeny from breeding groups in unsuitable breeding regions were more closely related to one another than were progeny of breeding groups in optimal regions. Changes were also observed in the genetic structure of Black and White cattle propagating within a 10-km zone of the Chernobyl accident; their genetic structure differed from the typical genetic pattern for this breed such that it appeared to shift to one that was more characteristic of beef cattle. Contributions of original genetic structure and the influences of artificial and natural selection on specific breed traits of genetic structure were examined.

P22 Isolation, morphology, and manipulation of primary oocytes from the turkey ovary

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A model system using yolkless oocytes (YO) is being developed to study sperm nuclear decondensation (SND) and events associated with oocyte maturation, fertilization, and embryogenesis. After incubation in trypsin-EDTA, YO ranging in diameter from 70 to 800 μm were released from cortical tissue. Morphological features varied with the diameter of the YO. For example, the germinal vesicle became difficult to discern in YO >300 μm because of the redistribution of lipid to the periphery of the YO. All YO were enveloped by a single investment of tightly apposed granulosa cells resting on a defined basement membrane. Consequently, micropuncture of this investment was successful only after considerable physical distortion of YO. Further enzymatic digestion of the granulosa investment rendered the denuded YO susceptible to damage during micropuncture. Microinjection of fluorescent-labeled intact sperm into YO <200 μm in diameter revealed no SND after 24 hours of incubation. Future studies include the microinjection of isolated sperm nuclei plus cytoplasm isolated from the blastodisc of mature oocytes after germinal vesicle breakdown into YO of different diameters.

P23 Characterization of a flow-sorted swine chromosome-6 library

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Swine chromosome-6 microsatellite markers were developed with libraries generated with size-fractionated DNA isolated from chromosomes sorted by flow cytometry. Chromosome isolation procedures were established to prepare high-quality chromosomes from PHA-stimulated swine lymphocytes and to sort individual chromosomes after staining with Hoechst 33258 and chromomycin A3. Chromosome purity was verified by FISH, and DOP-PCR generated probes showed specific staining of swine chromosome 6 from a chromosome-6 sort. The DNA was extracted from flow-sorted chromosome 6 and ligated into pBluescript SK II+. Cloned DNA was PCR amplified; 200 to 700 bp DNA fragments were isolated and subcloned into λZAP Express or pBluescript SK II+ to produce chromosome-6 libraries. The libraries were screened with a radiolabeled poly-d(CA) DNA probe; positive clones were sequenced. Only four of six potential microsatellite sequences proved successful by PCR (S0441, S0442, S0443, S0444). Chromosomes were mapped using U.S. Meat Animal Research Center and PiGMaP reference populations; linkage was analyzed using CRIMAP. Markers S0443 and S0444 mapped to swine chromosome 6, S0441 to chromosome 2, and S0442 to chromosome 8.

P24 Frequency of RFLP markers of the major histocompatibility complex class IV for experimental broiler lines divergently selected for early immune response

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An RFLP analysis was successfully applied to a meat-type chicken population using PvuII- or BglII-digested DNA hybridized with cDNA probe 32.1 of the major histocompatibility complex (MHC) class IV region. Identification of each haplotype was based on the pattern of 4 to 10 bands ranging from .8 to 8.0 kb. Divergent selection on early immune response of chicks has been conducted for five generations. Chicks were immunized by *Escherichia coli* vaccine at 10 days of age. Antibody titer was determined by ELISA 10 days later. A marked difference in antibody titer and in frequency of several RFLP bands and haplotypes of the MHC class IV region was found between lines divergent for anti-*E. coli* response. To ensure the direct effect of these RFLP markers, fifth-generation birds with high and low anti-*E. coli* response were crossed, and the MHC class IV haplotypes of their F_1 offspring were determined by routine RFLP analysis. Heterozygous F_1 males and females were mated to produce F_2 families. The F_2 response to vaccination was determined. These data facilitated unbiased estimation of the MHC class IV haplotype effect on immune system functions.

P25 Use of microsatellites in rare breed preservation

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In cooperation with ÖNGENE, an Austrian organization to preserve rare breeds, results of blood group testing are being used to develop genetic markers. One of the first projects was with Waldviertler Blondvieh (WB), the oldest cattle breed of lower Austria. During the last 30 years, WB have been replaced by Simmentals because Simmentals have higher milk yield. In 1972, the WB population consisted of 212 animals. In 1989, only 108 animals carried the WB marker genes (based on the B blood group system). Research has been extended by the use of molecular genetic methods, and the microsatellites ILSTS005 and CYP21 were tested for WB and Simmentals. No specific WB allele that could be used as a marker has been found yet. However, these two microsatellites can be used for parentage testing and in breeding programs. A similar project to preserve the Austrian horse breed Noriker has been started.

P26 Improved efficiency of Beltsville Sperm Sexing Technology using dual staining with Hoechst 33342 and propidium iodide

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Flow cytometric DNA sorting of X and Y chromosome-bearing viable sperm is well established and has been used to produce offspring of the predicted sex for cattle, sheep, swine, and rabbits. This procedure has also proven effective for separation of human X and Y sperm. The principle of dead-cell gating using propidium iodide (PI) or trypan blue (TB) in combination with Hoechst 33342 and single-laser ultraviolet excitation was applied to flow sort viable mammalian sperm. Dead sperm are gated out of the sort to allow the viable X/Y sperm (3 to 4% DNA difference) to be sorted more efficiently. With a more homogenous population, sorting speed was increased by $\geq$50% and sort purities by as much as 15%. In vitro viability assessment (motility) following sorts for which PI or TB were used was not different from Hoechst 33342 alone. The effects of these stains together on fertility has not yet been determined. This method of eliminating the dead cells holds promise for increasing the overall efficiency of viable X/Y sperm sorting.

P27 Expression of jet-injected plasmid DNA in the ovine mammary gland and in lymph nodes draining the injection site

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A jet-injection-based DNA delivery system was evaluated as a means for transient transfection of lactating mammary gland in vivo and as a technique for DNA vaccination. The model expression plasmid contained the human growth hormone (hGH) structural gene driven by the human cytomegalovirus immediate early gene 1 promoter/enhancer region (CMV). Expression from the naked plasmid DNA jet injected into sheep lactating mammary glands was sufficient to be detected by Northern blot analysis if tissue was obtained 48 hours after in vivo transfection. In contrast, mRNA expression following DNA transfer by needle and syringe was detectable by RT-PCR but not by Northern blot analysis. Specific mRNA could be detected by RT-PCR in lymph nodes draining the mammary gland injection sites. In a second experiment, time course and magnitude of antibody development to hGH following jet injection of CMV-hGH into either muscle or mammary gland were similar. Ability for transient transfection of lactating mammary tissue in vivo circumvents difficulties encountered with in vitro culture techniques and provides a method for examining mammary regulatory elements and testing fusion gene constructs designed for production of transgenic large animal bioreactors.

P28 Application of the single-strand conformation polymorphism method to the detection of microsatellite polymorphism

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Microsatellite markers have been extensively used for the construction of genetic linkage maps, which help in identification of genes for phenotypic trait such as QTL. Length polymorphism of microsatellite markers is generally detected by denaturation polyacrylamide gel electrophoresis of the microsatellites labeled with radioisotope or fluorescence in PCR. To detect microsatellite polymorphism without risk of irradiation and use of DNA sequencer, two systems were examined: 1) non-labeled PCR products separated on native polyacrylamide gels followed by staining with ethydium bromide and 2) analysis of single-strand conformation polymorphism (SSCP) followed by staining with silver nitrate. For the native gel system, polymorphism of microsatellites could be identified (even though complex band patterns were observed in the gel), and inheritance of the polymorphism could be deduced for most family individuals. The SSCP analysis, which is applied for detection of the base difference between DNA fragments, has detected each allele on all microsatellite loci examined. These findings indicated that SSCP analysis was more reliable than native gel analysis for detection of polymorphisms.

P29 Development of bovine genetic markers using temperature gradient gel electrophoresis

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Temperature gradient gel electrophoresis (TGGE) can detect single base mutations in DNA fragments by differences in electrophoretic mobility through a gradient of increasing temperature. A first TGGE attempt involved a 100-bp fragment (XT27) cloned from microisolated bovine X-chromosomal DNA. This fragment, which was length monomorphic in IBRP parents, revealed three alleles by TGGE after amplification with a GC clamp. This locus was genotyped across the IBRP and assigned to the bovine X chromosome. Primer pairs were also designed to amplify segments of the 3′-untranslated regions of several known bovine cDNA sequences. In each pair, one primer contained a 25-nucleotide GC clamp at its 5′ end. The IBRP parents were examined for polymorphisms at these loci using TGGE. Type II collagen pro-α1 chain (COL2A1), terminal deoxynucleotidyltransferase (DNTT), basic fibroblast growth factor (FGFB) and monoamine oxidase (MAOA) were polymorphic with three, three, two, and two alleles, respectively. The COL2A1 locus has been genotyped across the IBRP and mapped to bovine chromosome 5. Genotyping of BFGF, DNTT, and MAOA is in progress. These polymorphic loci can serve as anchored reference markers for comparative genetic analysis and can be used for studies analyzing QTL.

P30 Molecular cloning of a genomic fragment containing a glucose transporter gene and its assignment to a swine chromosome by in situ hybridization

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Glucose is the main energy source of mammalian cells and is efficiently delivered to all cells in the mammalian body (e.g., to brain cells through the blood-brain barrier). Uptake of glucose by most mammalian tissues is mediated by Na^+-dependent and facilitative glucose transporters. Facilitative glucose transporters constitute a gene family (GLUT1 to 5). Among these genes, GLUT1 and GLUT3 are expressed in brain cells and regulated by a different mechanism. The cosmid clone containing the GLUT1 gene was selected from the cosmid library of swine genomic fragments by colony hybridization using cDNA of swine GLUT1 gene (donated by B. Mockel, Technische Hochschule Darmstadt) as a probe. Southern blot analysis using several restriction enzymes on the cloned fragment with the genomic DNA suggested that the genomic fragment clone contained the GLUT1 gene. The cloned fragment was partially sequenced to compare with cDNA used for the probe and then with the rat genomic sequence of GLUT1, which revealed that the cloned genomic fragment contained at least part of the GLUT1 gene. Using the cloned fragment as a probe, FISH was performed on swine-chromosome spreads. The fluorescence signals demonstrated that the GLUT1 gene is located at swine chromosome 6q34-qter.

P31 Association of major histocompatibility complex class II DNA polymorphisms and Marek's disease resistance in chicken lines selected for multitrait immunocompetence

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Association of the major histocompatibility complex (MHC) and Marek's disease (MD) resistance in inbred and commercial chicken lines has been well documented. Previous reports used serological definition of MHC and did not provide direct information on MHC class II genes. Association of MHC class II DNA polymorphisms and MD resistance was investigated using herpes virus turkey-vaccinated and unvaccinated chickens. The four White Leghorn lines were divergently selected for multitrait immunocompetence based on cellular, humoral, and phagocytic responses. Thirty chicks per line per vaccination treatment (240 total) were challenged with highly virulent MD virus (Md5, 500 FFU) at 2 days of age. Morbidity, mortality, and tumors in nerves and visceral organs were recorded daily and at necropsy at 10 weeks. Samples of PvuII-digested, electrophoresed genomic DNA were hybridized with chicken MHC class II probe. Nine high-intensity bands (2.1 to 6.5 kbp) were observed. There was an interaction of vaccination treatment and MHC class II DNA polymorphism association with MD resistance. There were MHC class II DNA polymorphisms in multitrait immunocompetence lines that were associated with tumor and MD incidence for both the vaccinated and unvaccinated treatment groups. Specific MHC class II bands were associated with tumor development and MD resistance.

P32 Variants of DNA within the 5′-flanking region of the porcine follicle-stimulating hormone β gene

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To detect potential sequence variations among pig breeds, the 5′-flanking region of the follicle-stimulating hormone β gene from Erhualian, Meishan, Yorkshire, Landrace, and Xiang pigs were cloned and sequenced. Two single-bp deletions and two pyrimidine-to-pyrimidine substitutions were identified within the 5′-flanking region from +494 to +1000 for Erhualians and Yorkshires. Only a single purine-to-purine substitution at position +421 and a dramatic AT simple-repeat variant at position +366 were located within the 5′-flanking region from +3 to +493 among the five breeds. No DNA variants were found in potential binding sites for *trans*-acting factors or in the 5′-untranslated region of exon I.

P33 Differential expression of bovine β-lactoglobulin A and B, promoter differences, and binding affinity of transcription factor AP2

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The molecular nature of observed quantitative differences in milk protein expression was studied for allelic variants A and B of β-lactoglobulin (β-LG). The proximal region of the β-LG promoter located 780 bp upstream from the transcription start site was evaluated. Using denaturing gradient gel electrophoresis and sequencing analysis, seven point mutations were detected within the distal 450-bp region of the promoter. All mutations except one were found in only one of the two alleles. The most interesting point mutation was a G-to-C transversion in the AP2 transcription factor binding site at position −476 from the transcription initiation site. Mobility shift assay demonstrated a differential binding affinity of AP2 for both allelic variants. Promoter variant A was able to displace variant B from the AP2 protein. Footprinting assay with deoxyribonuclease I clearly revealed a protected AP2 binding site in variant A (but not in variant B) with a mutated AP2 site. These results suggest significant differences in the binding affinity of AP2 between the promoter variants with possible influence on the mRNA synthesis rate causing differential expression of β-LG A and B alleles. Evaluation of both promoter types in an in vitro transcription system using luciferase reporter gene mediated transient expression is underway.

P34 A male linkage map of the cattle genome

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A male linkage map of the cattle genome was constructed using nine large paternal half-sib families of the Illinois Reference/ Resource Families (IRRF). The map consists of 225 microsatellites and 20 structural genes. Genome coverage is ~2000 cM on 28 autosomes and one unassigned linkage group. Of the microsatellites, 128 are anchor markers, and 97 are newly assigned to 25 autosomes. Because anchor markers were selected from Meat Animal Research Center and IBRP maps, the three maps were integrated to facilitate developing a consensus linkage map. The large number of male meioses permitted development of an accurate sex-specific map and resolution of ordering problems in other maps. Map agreement for genetic distances between anchor markers was excellent, but several discrepancies were found. The IRRF map is being used to search for QTL in these families. Development of an accurate male linkage map should aid marker-assisted selection through the sire path and permit greater precision in map-based cloning strategies. The map is sufficiently dense for several sires to permit assignment of new markers by meiotic breakpoint mapping.

P35 Evaluation of the locus for cysteine-rich intestinal protein as a candidate gene for Adema disease of cattle

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Adema disease is an autosomal recessive disorder that results in absorption of inadequate amounts of zinc from the gastrointestinal tract and leads to a number of clinical abnormalities. For rodents, a cysteine-rich intestinal protein (CRIP) has been identified as a zinc transport protein. A mutation at the CRIP locus was hypothesized to be responsible for Adema disease of cattle. A pedigree was established using two cows that were obligate heterozygotes as embryo donors and semen from an affected bull in AI and embryo transfer studies to obtain seven offspring. The first clinical manifestation of zinc deficiency was diarrhea, followed by skin lesions and a decreased ability to suckle. Oral administration of zinc in pharmacological amounts reversed all signs and symptoms. Three-fourths of the coding region of the bovine CRIP locus has been isolated and sequenced using molecular genetic techniques; however, no significant differences between normal and affected animals have been found yet. The CRIP locus may appear intact in its coding region but have a mutation in its flanking regions that affects its expression. A linkage study is being conducted with the pedigree to provide a formal test of CRIP as the site of the mutation.

P36 An updated map of the porcine genome based on a wild pig × Large White cross

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A primary porcine linkage map based on 128 genetic markers was updated to include 110 additional markers. New assignments include 12 RFLP, 2 single-strand conformation polymorphisms, 4 blood groups (EAD, EAE, EAK, and EAO assigned to chromosomes 12, 9, 9, and 6, respectively), and the extension coat color locus (chromosome 6). Esterase D (ESD) was assigned to chromosome 11, which conflicts with previously reported in situ assignment of ESD to chromosome 13. One previously unassigned linkage group, EAK-HPX, was assigned to chromosome 9. The linkage map covers about 2170 cM, and mean distance between markers was 11 cM. Based on total recombination distance, including 71 markers physically anchored by in situ hybridization in previous studies, the sex-average genome length was estimated at 2400 cM ± 270 (SE_{mean}). Significantly higher female than male recombination was found for 13 autosomes. This comprehensive map will be utilized for mapping QTL segregating in the wild pig × Large White cross.

P37 Forensic identification of horse urine samples using nuclear and mitochondrial DNA markers

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The possibility of verifying the identity of urine samples involved in horse doping cases was investigated with urine samples from 12 horses using nine microsatellite markers and one mitochondrial D-loop single-strand conformation polymorphism. Genotypes were detected by PCR analysis using a simple protocol for DNA preparation from different urine volumes corresponding to 5, 40 and 80 μl of urine per reaction. Urine and blood samples showed identical genotypes for all horses. However, for a few cases, the PCR analysis of the smallest urine volume per preparation did not reveal more than one of the two alleles in a heterozygote genotype. For these cases, the two alleles were randomly amplified, probably because of an extremely low number of initial target molecules. For most cases, the amount of PCR product increased with increasing volume per preparation, but three samples showed a drastic reduction of PCR product. For these samples, ultrafiltration of the most concentrated preparation dramatically improved the results and enabled genotyping with all markers. These results show that the methods used are highly valuable for identity testing of horse urine samples with an estimated total probability of identity by chance $<10^{-7}$ for Standardbreds.

P38 Effect of pronuclear DNA microinjection on in vivo development of porcine ova

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Early development of DNA-microinjected and noninjected porcine ova in utero were compared. Seventy-five sexually mature gilts were fed allyl trenbolone (AT) for 14 to 16 days. Forty-eight hours after the last day of AT consumption, gilts received pregnant mare serum gonadotropin followed 80 hours later by human chorionic gonadotropin (HCG). Donor gilts were bred at 24 and 36 hours after the onset of estrus. Ova were recovered between 60 and 62 hours after HCG administration. Fertilized 1-cell ova were randomly allocated from each donor across two treatments: 1) pronuclei were injected with genes that code for the human complement regulatory proteins decay accelerating factor and membrane cofactor (MICRO) and 2) no microinjection (CONTROL). Ova were transferred to oviducts of recipients that had exhibited estrus on the same day as donors. Ova were recovered after 60 or 120 hours in utero and stained with 1% orcein. The CONTROL ova possessed significantly greater numbers of nuclei than MICRO ova recovered after 60 or 120 hours in utero. The percentage of ova that formed blastocysts and the mean number of nuclei present in blastocysts were also significantly greater for CONTROL ova compared with MICRO ova. These results demonstrate that pronuclear DNA microinjection reduces early development of porcine ova in utero.

P39 In vitro culture of porcine inner cell mass: effect of culture medium and human leukemia inhibitory factor on differentiation

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Isolation of porcine embryonic stem (ES) cells has been hindered by inability to inhibit differentiation of the porcine inner cell mass (pICM) in vitro. Current culture conditions were developed from mouse ES cell culture. Optimizing culture conditions for pICM is essential. A differentiation grading system was developed to detect changes in status of in vitro cultured pICM. The pICM (day 7) were isolated by immunosurgery and cultured for 4 days in DMEM-based medium (D medium) or DMEM/Hams F-10 (1:1)-based medium (D/H medium) with or without human leukemia inhibitory factor (hLIF, 1000u/ml). Colonies were photographed daily for morphologic analysis. The pICM were categorized into two development patterns: 1) round ES-like or 2) flat epithelial-like. Eight investigators evaluated pICM differentiation using standardized differentiation profiles. Each pICM series was graded on a scale of 1 (completely undifferentiated) to 5 (completely differentiated) for each time point. Neither hLIF nor culture medium delayed differentiation of pICM. The grading system employed was an effective tool for detecting treatment effects on differentiation of developing pICM. These results demonstrate that hLIF cannot significantly inhibit differentiation of the pICM and is unlikely to assist in isolation of porcine ES cells. Future experiments with homologus cytokines may prove more beneficial.

P40 Physical mapping of complex traits in an arthropod vector of animal diseases: *Culicoides variipennis* and the bluetongue viruses

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A recombinant cosmid library is being used as probes in FISH physical mapping of *Culicoides variipennis*, the primary vector of bluetongue virus in North America. Metaphase chromosomes were prepared from a *C. variipennis* cell line (CuVa). Probes were nick-translated with biotin-11-deoxyuridine triphosphate using standard protocols. To eliminate the confounding effects of CuVa DNA repetitive sequences, suppression hybridization with a CuVa Cot-1 DNA fraction was necessary. The specific hybridization was detected with avidin-fluorescein isothiocyanate (FITC). Slides were counterstained with diamidino-2-phenylindole (DAPI). The DAPI- and FITC-stained digital images were captured with a cooled-array, charge-coupled device collector mounted on a Zeiss Axioskop microscope. Digital images were enhanced, colorized, and merged, and chromosomal lengths and features were measured. A physical map of the *Culicoides* genome will be constructed to enable mapping of loci that control vector competence for bluetongue virus. Specific probes for vector competence loci will allow monitoring of vector populations and assessment of their potential as vectors for bluetongue virus and related, introduced pathogens.

P41 Updated genetic linkage map of porcine chromosome 6

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Linkage analysis of quantitative traits and markers included in a genetic map aim for identification of specific markers linked to the trait of interest. The selected strategy for gene mapping of animals includes the use of reference populations and informative, highly polymorphic genetic markers. Initial detection of linkage between several markers mapped to pig chromosome 6 and genes affecting growth and carcass traits of pigs has been reported. Initial mapping efforts have concentrated on the construction of the chromosome-6 genetic linkage map based on the University of Illinois Meishan × Yorkshire reference population. The database (334 animals) includes measurements for 70 growth, carcass, and reproductive traits. The current genetic map of chromosome 6 is based on genotypes for five type I (RFLP) and eight type II (microsatellite) markers. Three additional microsatellites (JM3.1.1, JM3.1.2, and JM3.1.3) were recently identified and included on the map. Current goals include construction of the genetic linkage map for the whole genome based on 80 highly polymorphic microsatellites and genome scan for QTL.

P42 Changes in gene expression during retinoic acid-induced differentiation of murine embryonic stem cells

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Analysis of gene expression changes during differentiation of murine embryonic stem (ES) cells and porcine embryo-derived cell lines has been hampered by the inability to measured quantitative gene expression changes from small tissue samples. Reverse transcription-T7 RNA polymerase dependent amplification (RT-TRDA) has been proposed as a technique that can be used to measure quantitative changes in gene expression of multiple genes simultaneously. To ensure that gene expression profiles observed with RT-TRDA agree with the existing mRNA population of the analyzed cells, the pattern of gene expression of undifferentiated and retinoic acid-induced differentiated murine ES cells were analyzed by RT-TRDA and/or Northern and RNA protection assays. Results indicate that several parameters of the original RT-TRDA technique produce less than reliable results compared with Northern or RNA protection assays. Preliminary results with a modified RT-TRDA procedure indicate that this modified procedure produces a close match to results obtained with Northern or RNA protection assays.

P43 Germline transmission of immunoglobulin transgenes conferring resistance to pathogenic *Escherichia coli* in mice

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To induce constitutive immunity against pathogenic *Escherichia coli* that induce scours in animals, transgenic mouse models were created. Rearranged immunoglobulin (Ig) genes were isolated that targeted a K99 pilus antigen. Chimeric Ig heavy chain (HC) constructs composed of a $\mu\kappa$ enhancer/promoter, hybridoma and PCR-derived VDJ region, and bovine α constant region were cloned. The HC3 clone was injected into 384 zygotes; 3 of 42 founders were transgenic. Transgenic founders expressed HC3-specific mRNA and protein, were protected against pathogen gavage, but HC3 germline transmission was not obtained. The HC3 construct was recloned (designated HC4) and reinjected into 250 zygotes; 34 founder mice were born, 5 of 24 weaned founders were transgenic, and 4 of 4 bred founders demonstrated HC4 germline transmission. Two adult and two preweaning offspring from line 4-1 and from line 11-1 were subjected to a K99 crude extract challenge. A K99 VDJ probe detected mRNA transcripts in individual spleen samples obtained 24 hours postchallenge from all HC4 transgenic mice but not from littermate or age-matched controls. Use of the HC4 construct resulted in increased transgenic yields and germline transmission of the transgene, with HC4 mRNA detected in adults and preweaning (immunoincompetent) offspring. Engineering pathogen resistance provides a timely application of transgenic technology beneficial to production agriculture and to the safety of the food supply.

P44 Screening by PCR for carriers of bovine leukocyte adhesion deficiency and deficiency of uridine monophosphate synthase in Argentine Holstein cattle

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Bovine leukocyte adhesion deficiency (BLAD) and deficiency of uridine monophosphate synthase (DUMPS) are monogenic autosomal recessive inherited diseases of Holstein cattle. A PCR-based assay was used to detect point mutations responsible for the genetic disorders. Using oligonucleotide primers, DNA fragments of predicted sizes were amplified, and product specificity and mutation identity were assessed by nucleotide sequencing. Mutations were detected in DNA samples from bovine blood and semen by presence or absence of a restriction site within TaqI for BLAD and AvaI for DUMPS. Tests included 104 Argentine Holstein bulls and 950 cows. Defective allele frequencies were 2.88% BLAD for active AI bulls and 1.79% for cows and .96% DUMPS for active AI bulls and .10% for cows. Assuming random mating, <.052% of matings would be between carriers, but such matings would yield 25% BLAD homozygous offspring (290 dead calves each year). Mating carrier bulls with normal cows would yield 31,820 heterozygous carrier calves. Although frequency of the defective alleles is low, monitoring herd and active AI bull genotype is necessary to avoid dissemination of these genetic disorders.

P45 Identification of QTL for reproduction traits in mice

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The mouse genome was systematically screened to identify chromosomal regions harboring QTL that control reproduction traits. An F_2 intercross was produced originating from the long-term selection lines M16i (rapid weight gain) and L6 (small body weight). Parental lines diverged greatly for ovulation rate (OR), embryo survival (ES), and litter size (LS). The F_2 females (445) were mated at 10 weeks and evaluated at day 17 of gestation. Genotypes were identified at 66 autosomal microsatellite markers at intervals of 20 to 30 cM. Markers indicating QTL with significant additive effects were found on chromosomes 3, 8, 11, 13, 18, and 19 for OR; 2, 9, 10, 11, and 19 for ES; and 2, 10, and 19 for LS. The QTL with the largest additive effect was found on chromosome 2, accounting for a difference of 13.6% in ES and an advantage of 2 live pups for M16i/M16i females. Other additive effects were in the range of .8 to 1.0 ova for OR, 7.2 to 11.5% for ES, and 1.3 to 1.5 live pups for LS. Markers potentially linked to QTL with significant dominant effects were found on chromosomes 6, 12, and 16 for OR; 1, 2, and 13 for ES; and 1 and 2 for LS. Future research will attempt to identify the genes and to locate their homologues for livestock species.

P46 Xenografts of livestock embryonic cells into mice with severe combined immunodeficiency

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Conditions were examined for derivation of embryonal carcinoma (EC) cell lines and teratoma or teratocarcinoma formation from farm animal embryos in mice with severe combined immunodeficiency (SCID). Day-7 blastocysts and day-13 embryonic discs (ED) from pigs, sheep, and cows were surgically implanted in the kidney capsule, testis, and shoulder of SCID mice. For controls, D3 mouse stem cells and STO mouse fibroblasts were injected at 1×10^6 cells/site. After 6 weeks, mice were examined for tumors, which were then cultured to isolate EC cells, tested for species identity by PCR, and examined histologically. The D3 and STO cells produced tumors at all three sites for all five mice. Day-7 blastocysts produced no tumors from cows (60 sites, 4 mice) or pigs (20 sites, 4 mice) and 2 tumors from sheep (22 sites, 3 mice). Day-13 ED produced 9 tumors from pigs (31 sites, 10 mice) and 8 tumors from sheep (28 sites, 10 mice). A total of two shoulder, nine kidney, and eight testicular tumors from injections of embryonic tissue were found. In tissue culture, all tumors yielded fibroblasts, macrophages, and epithelial cells. Some tumors contained hair, bony fragments, neuronal cells, muscle fibers, and ciliated epithelium. Using species-specific primers, PCR identified all tumors as containing either pig or sheep tissue. The SCID mouse xenografts may be useful for testing putative ES cell lines and may provide a means of deriving EC cells for farm animals.

P47 Methylation of DNA and βCasLacZ gene expression in transgenic rabbits

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Transgenic rabbits could be a convenient model for investigating expression of heterologous proteins in mammary glands. Transgenic rabbits with the βCasLacZ gene construct were generated. Some 2096-pb upstream sequences of the rabbit β-casein gene were fused in pCH110 plasmid to the 3.5-kb *Escherichia coli* LacZ reporter gene and SV40 polyadenylation site. Transgene integration was detected by Southern blot and PCR analyses. Although some ectopic expression of the LacZ gene was detected in liver and spleen, no mammary-gland expression was shown for transgenic lactating rabbits by Northern blot analysis. Lack of expression might have resulted from transgene hypermethylation. Heavy methylation of C residues in the LacZ gene was shown upon restriction analyses with methylation-sensitive MspI and HpaII nucleases. Transgene expression was detected in mammary gland explants derived from pregnant transgenic rabbits cultured for 2 days in the presence of lactogenic hormones (insulin, hydrocorticsone, and prolactin) and 5-azacytidine, a demethylating agent. Expression of a transgene-bearing milk protein gene promoter might be regulated by its methylation; in some experiments, hypermethylation might be a cause of the lack of transgene expression.

P48 Use of microsatellites and a bovine interspecific hybrid backcross panel to orient linkage maps on bovine chromosomes 2 and 28

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To refine linkage maps of bovine chromosomes 2 (BTA 2) and 28 (BTA 28), a *Bos taurus* × *Bos gaurus* interspecific hybrid backcross (IHB) panel and polymorphic microsatellite markers were used to orient previously published maps physically. The gaur (2n=58) differs karyotypically from cattle only by the presence of a 2;28 Robertsonian translocation. Because interspecific hybrid females used in the backcrosses are heterozygous for the translocation chromosome, the translocation served as a centromeric marker for both chromosomes. The IHB panel was screened with microsatellite markers chosen from each end of published BTA 2 and BTA 28 linkage maps. On BTA 2, no recombination was observed between the centromere and TGLA431, whereas 56% recombination was observed for FN1. The loci BM4440 and INHA were not informative. On BTA 28, 33% recombination occurred between the centromere and RM16, and 37.5% recombination was observed for BM2515. The loci RBP3 and BL25 were not informative. Markers near the telomere are expected to recombine more frequently than those tightly linked to the centromere. Thus, preliminary data indicate that BTA 2 is oriented centromere-TGLA431-FN1-telomere. Data for BTA 28 remain inconclusive, and additional markers for this chromosome are being analyzed.

P49 Strategies for identification of genes controlling the vector competence of *Culicoides variipennis* for bluetongue virus

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Markers for DNA, simple sequence length polymorphisms, RAPD, and single-stranded conformation polymorphisms have been identified for generation of a genetic map of *Culicoides variipennis*, the North American insect vector of bluetongue (BLU) viruses. Traits associated with vector competence vary among insects. This variation is likely a result of multiple genes and environmental factors. Strategies for identification and cloning of these quantitative trait genes using molecular markers were investigated. Molecular markers will be used in recombination mapping to assign vector competence genes for BLU virus to their chromosomal locations. Molecular markers will then be used to identify deletion mutants and clone vector competence genes.

P50 Cre-mediated deletion at a targeted mouse whey acidic protein locus

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A mouse genomic DNA library prepared from the embryonic stem (ES) cell line E14gT2a was screened for the whey acidic protein (WAP) gene. The DNA from one positive phage clone digested to give a 4.5-kb XhoI-EcoRI fragment containing all four WAP exons was subcloned into Bluescript. To generate the targeting construct, a neomycin-thymidine kinase (Neo-TK) cassette flanked by two 34-bp loxP sites was positioned in exon 4 of this subclone. Using this construct, AB1 ES cells were electroporated and placed under G418 selection. Positive clones were analyzed by genomic Southern blot to identify targeted clones. A positive cell line was expanded and electroporated with a cre-encoding plasmid to cause the deletion of the Neo-TK cassette. The ES cells containing a single loxP site were selected in ganciclovir-containing medium and identified by Southern blot analysis. The loxP-tagged ES cells will be used for in vivo studies to test the efficiency of cre-mediated recombination at the mouse WAP locus. The effects of incoming DNA size on efficiency and accuracy of recombination will be determined from coelectroporation of foreign DNA containing a second loxP site and the cre enzyme.

P51 In vivo analysis of muscle metabolism by ^{31}P nuclear magnetic resonance spectroscopy in pigs of different porcine stress syndrome genotypes

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Spectroscopy with ^{31}P nuclear magnetic resonance was performed on 4- to 9-week-old pigs to provide basic metabolic information for halothane-induced malignant hyperthermia as a part of porcine stress syndrome. The ryanodine receptor gene test determined the homozygous (NN) nonsusceptible pigs (24) and heterozygous (Nn, 29) and homozygous (nn, 16) stress-susceptible pigs. Changes of adenosine triphosphate (ATP), inorganic phosphate (Pi), phosphocreatine (PCr), pH values, and body temperature were observed 10 minutes before, during, and 60 minutes after halothane administration. Spectra were acquired every minute by placing a surface coil above the biceps femoris muscle. The nn genotype showed a more dramatic drop in PCr, pH, and ATP and a higher increase in Pi and body temperature after only about 8 minutes of halothane exposure than did the Nn and NN pigs, which were exposed to halothane for an average of 49 minutes. In the NN pigs, the muscle metabolism altered very slowly or did not change. The heterozygous genotype showed the largest variation in its in vivo response to halothane but tended to be more like NN than nn. The differences among the three genotypes (with an intermediate response by the heterozygous genotype) became more obvious by including post mortem measurements.

P52 Cloning and expression of the porcine and bovine leukemia inhibitory factor gene

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In spite of repeated attempts, isolation of stable pluripotential porcine or bovine embryonic stem (ES) cells has not been possible in the presence of mouse leukemia inhibitory factor (LIF), human LIF, STO feeder cells, or homologous and heterologous embryonic fibroblasts. To determine whether recombinant LIF may be more effective in inhibiting the differentiation of porcine or bovine ES cells, the LIF genes from both species were isolated. The porcine LIF was isolated from a genomic library using human LIF as a probe. A positive phage was subcloned into a plasmid, partially sequenced to confirm identity, and modified for expression in Cos-7 cells. The bovine LIF was isolated from a spleen cDNA library using an exon-3 probe generated by PCR. A positive clone is being expanded for characterization and cloning into an eukaryotic expression vector. Once isolated, the recombinant proteins will be used to determine their ability to assist in the isolation and maintenance of stable pluripotential ES cell lines.

P53 An assorted set of DNA markers for developing a preliminary genetic map of the turkey

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Using diverse molecular analysis techniques, markers are being developed for mapping the turkey genome, an agriculturally important livestock species that is among the least understood. The panel used to test the inheritance pattern of the markers is based on a pedigree established from a commercial turkey × wild turkey reciprocal cross. The approaches used to identify the markers included RAPD, RFLP, and microsatellites. In the RAPD approach, 60 random primers have been screened. Analysis by RFLP has involved heterologous probes based on the chicken genome (gift of Dr. N. Bumstead) and random cDNA clones from a turkey pituitary cDNA library. In the microsatellite approach, several enriched libraries have been established for $(TG)_{14}$, $(GAT)_7$, $(GATA)_5$, and $(CTA)_7$. From these libraries, positives based on a double screen and Southern blot analysis are being sequenced. Additionally, primers specific for amplification of (TG) or (CA) in chickens have also been used. Hopefully, a preliminary linkage map will facilitate both comparative mapping in the turkey and subsequent identification of markers for QTL, including those for reproductive efficiency.

P54 Lipid composition of carcass tissue from transgenic pigs expressing either a bovine, ovine, or human growth hormone gene

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Fatty acid profiles and cholesterol content of ground whole-carcass tissue from transgenic (T) pigs expressing either a bovine (bGH), ovine (oGH), or human (hGH) growth hormone gene were compared with those from sibling control (C) pigs. All pigs were fed a common growing diet and were slaughtered at approximately 100 kg live weight. The left side of each intact carcass was ground and used for whole-carcass evaluations. All samples were analyzed for lipid composition and cholesterol content. Carcasses from TbGH pigs contained 84% less fat (27.5 versus 4.5%), which was 87% less in saturated fatty acids (SFA), 89% less in monounsaturated fatty acids (MUFA), and 64% less in polyunsaturated fatty acids (PUFA), than C-pig carcasses. Carcasses from ToGH pigs contained 82% less fat (27.5 versus. 4.8%), which was 85% less in SFA, 85% less in MUFA, and 72% less in PUFA, than C-pig carcasses. Carcasses from ThGH pigs contained 64% less fat (27.5 versus 9.9%), which contained 70% less SFA, 68% less MUFA, and 37% less PUFA. Cholesterol content of whole-carcass tissue was not different between T- and C-pigs. These results suggest a major reduction in total lipid and fatty acid content in carcasses from transgenic pigs compared with controls.

P55 Fluorescence-activated cell sorting of transfected chicken blastodermal cells prior to injection into recipient embryos

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Construction of transgenic chickens would facilitate studies of genetic control of development, and genetic sequences encoding traits of agricultural and pharmaceutical importance could be incorporated into the genome of these birds. Transgenic chickens could be constructed through chimeric intermediates by injecting genetically manipulated donor cells into recipient embryos such that some of these cells contribute to the germline of the resulting chick. The chance of producing a transgenic chimera would be increased by enriching the donor population in transfected cells. To demonstrate that chicken blastodermal cells can be sorted according to expression of a specific gene, cells in suspension were transfected with pmiwZ, a plasmid containing the lacZ gene encoding bacterial β-galactosidase (β-gal). Following overnight incubation, cells were loaded with 5-dodecanoylaminofluorescein di-β-D-galactopyranoside, which is fluorescent upon cleavage by β-gal. A single cell suspension was prepared and subjected to fluorescence-activated cell sorting. A large proportion of the sorted population showed expression of β-gal as demonstrated by staining with X-gal, a chromogenic substrate of β-gal. Sorted cells were successfully cultured on mouse SNL 76/7 fibroblast feeder cells, and formed colonies. Sorted cells were also injected into recipient embryos and were incorporated to form a chimeric embryo.

P56 Efficient cloning method to detect microsatellite markers in chickens

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Microsatellite markers are used increasingly in animal genome analysis; however, informative microsatellite markers are limited for chickens. Recently, some methods for cloning microsatellite sequences have been reported. A new method that is easy to use for cloning of microsatellite sequences was developed for chickens. Chicken genomic DNA fragments digested with four restriction enzymes were ligated into PCR-Script SK(+) vector. The ligation mixture was transformed into XL1-Blue MRF′ competent cells. Without the plating step, the cells were infected with helper phage, and single-stranded DNA was prepared by standard procedures except with a modification as follows. After the recombinant phage precipitation step, DNaseI and RNaseA were used to remove contaminating *Escherichia coli* DNA and RNA from the resulting single-stranded DNA in the phage mixture. The DNA was used as a template for double-stranded DNA synthesis, primed with $(CA)_n$ oligonucleotide, by thermostable DNA polymerase. Subsequently, single-stranded DNA remaining in the mixture was digested by mung bean nuclease, and then the mixture was transformed into XL1-Blue MRF′ competent cells. Transformants containing microsatellite sequences were recovered efficiently; thus, this method will contribute to genetic mapping for chickens.

P57 Transcription of leukemia inhibitory factor receptor-β and octamer-binding transcription factor-4 in preimplantation bovine embryos

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Transcription of leukemia inhibitory factor receptor-β (LIFR-β) and octamer-binding transcription factor-4 (OCT-4) was investigated in in vitro-produced preimplantation bovine embryos by RT-PCR analysis. Sets of nested oligonucleotides corresponding to the coding regions of these genes were designed based on consensus cDNA sequences of the human and murine LIFR-β gene and on a bovine genomic OCT-4 sequence; these sets were verified to span at least one intron to favor the amplification of cDNA over genomic DNA. Total RNA was isolated from oocytes, from embryos at the 2-, 4-, 8-, 16-cell, morula, and blastocyst stages, and from cells of the trophoblast and inner cell mass isolated by immunosurgery. The RT-PCR analysis resulted in specific amplification products of LIFR-β and OCT-4 in all samples tested (which demonstrated active transcription from the maternal and the embryonic genome) and following the first differentiation of embryonic cells. These results suggest that transcription of LIFR-β and OCT-4 is not restricted to totipotent cells of the preimplantation bovine embryo.

P58 Identification of molecular markers for strain evaluation of farm-raised channel catfish

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Production of channel catfish (*Ictalurus punctatus*) accounts for more than half of the U.S. aquaculture industry. Genetic selection is not commonly practiced within the industry, and the genetic integrity of commercial catfish strains is virtually unknown. Because the goal of the Catfish Genetics Research Unit is to develop genetically improved germplasm for release to producers, molecular genetic markers for strain verification and marker-assisted selection are being identified. Using primers designed from a carp mitochondrial genome, the mitochondrial D-loop region and 16SRNA gene have been PCR amplified for 65 families from 7 channel catfish strains and 2 families from one strain of blue catfish (*Ictalurus furcatus*). Amplified fragments were screened with 26 restriction enzymes for fragment length polymorphism. Fragment polymorphism fixed between channel and blue catfish was detected by eight enzymes in the D-loop region and three enzymes in the 16S RNA gene. Analysis of channel × blue hybrids confirmed maternal inheritance of the mitochondrial genome. Five enzymes revealed fragment polymorphism within channel catfish, although no fragment patterns were strain specific. Genomic DNA hybridized with multilocus probes has shown significant individual variation. Di-, tri-, and tetranucleotide repeat loci in the channel catfish genome also are being characterized.

P59 Chinese breeds of domestic animals for gene mapping

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China has a rich diversity of domestic animals. More than 200 Chinese native breeds of domestic animals have been documented. Notably, the Chinese Erhualian pigs are the most prolific pigs, and the Hu and Large-tailed sheep are prolific sheep breeds. The Xiang and Jinhua pigs, the Tong sheep, and the Beijing ducks are known for meat quality and flavor. Chinese pigs have not been found to have PSE and porcine stress syndrome genes. In recent years, Yellow-feathered chickens have made a comeback in the chicken meat market in China and among overseas Chinese because of their meat quality and taste. Their comeback shows the importance of understanding and conserving genetic diversities to meet changing consumer demands. The Silky is a medicinal breed and the only chicken breed known to have black skin, muscle, bone, and internal organ membrane. The genetic diversity of Chinese breeds is a rich resource of economically important genes and excellent raw material to map and to verify functional genes. Mapping and characterization of the economically important genes possessed by Chinese breeds should benefit animal breeders and consumers as well as global conservation of genetic resources and biodiversity.

P60 Genetic relatedness estimated by RAPD in lines of chickens and turkeys

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Genetic relatedness was estimated among three broiler lines (high, low, and control) for tibial dyschondroplasia incidence, two commercial layer stocks (DeKalb Delta and Hy-Line W-36), and two turkey lines (high and low) for blood pressure using RAPD from mixing equal amounts of DNA from 20 individuals within each line with 20-decamer oligonucleotide primers. Assays indicated a higher similarity between lines of the same breed (average band sharing = .75, .92, and .94 for the broiler, layer, and turkey lines, respectively) than between breeds (average band sharing = .63, .36, and .50 for the broiler versus layer lines, broiler versus turkey lines, and layer versus turkey lines, respectively). Similarities among the three broiler lines were lower than between either the two layer lines or the two turkey lines, which reflected a greater heterogeneity within the divergently selected broiler lines for tibial dyschondroplasia incidence used in this study. These results demonstrated that RAPD assay with mixed template DNA could be used for assessment of genetic relatedness among populations in poultry breeds and species.

P61 Characterization of recombinant chicken stem cell factor produced in mammalian and insect cells

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Stem cell factor (SCF) is a pleiotropic growth factor that is the ligand for Kit tyrosine kinase receptor. Studies on mouse *steel* mutants have shown that this locus encodes mouse SCF (mSCF), which is essential for the normal development of hematopoietic cells, germ, cells and melanoblasts. Later, mSCF was found to be expressed in the nervous system as well as the cell lineages as above, which suggests the possible role of SCF in the nervous system. To study the function of avian SCF, a chicken SCF (chSCF) cDNA was cloned and the nucleotide sequence determined. Recombinant soluble chSCF was formed in Cos-7 cells as a result of the processing of the cell-associated form. Truncated chSCF containing residues −25 to 170 was produced in Cos-7 cells and accumulated in the supernatant; this truncated chSCF had a similar apparent size to the soluble form derived from the intact protein. Truncated chSCF also was produced in insect cells using a recombinant baculovirus and was purified by column chromatography. The recombinant soluble chSCF induced the outgrowth of neurons from dorsal root ganglia of chicken embryos cultured in vitro. Presence of 50 ng of chSCF per milliliter of culture medium stimulated maximal neuronal outgrowth.

P62 Construction of a chicken bacterial artificial chromosome library

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Several lines of evidence suggest that bacterial artificial chromosomes (BAC) are useful as general cloning vehicles to construct comprehensive genomic libraries for a variety of organisms. The BAC system is suitable for high efficiency cloning of large DNA fragments (>300 kb) in *Escherichia coli*. A chicken genomic DNA library was constructed to be useful for physical mapping experiments and to allow the selection and characterization of Z chromosome-specific sequences. Mega-sized DNA was prepared from blood of a White Leghorn chicken. The DNA embedded in agarose plugs was partially digested with HindIII and size-fractionated through two successive rounds of pulsed-field gel electrophoresis to obtain fragments ranging in size from 200 to 500 kb. Ligation with the pBeloBACII vector was performed and followed by transfer of the recombinant plasmids into *E. coli* DH10B cells by electroporation. Blue-white colony selection was used to identify positive BAC clones, which were stored at −70°C. The library is being analyzed for insert size and stability, and specific clones are being selected by hybridization and PCR screening using a variety of Z-specific probes and primers. As part of a program to construct transgenic birds through chimeric intermediates, Z-specific sequences are required for construction of gene targeting vectors that will enable specific modifications of the chicken in a sex-linked fashion.

AUTHOR INDEX

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ABBREVIATIONS

AI	artificial insemination
ARS	Agricultural Research Service
B.A.	bachelor of arts
bp	base pair
B.S.	bachelor of science
cDNA	complementary deoxyribonucleic acid
DNA	deoxyribonucleic acid
DOP	degenerate oligonucleotide primer
EDTA	ethylenediaminetetraacetate
ELISA	enzyme-linked immunosorbent assay
FISH	fluorescence in situ hybridization
IBRP	international bovine reference family panel
M.S.	master of science
PCR	polymerase chain reaction
PHA	phytohemagglutinin
Ph.D.	doctor of philosophy
PSE	pale, soft, and exudative
RAPD	random amplified polymorphic DNA
RFLP	restriction fragment length polymorphism
RNA	ribonucleic acid
RT	reverse transcriptase/transcription
QTL	quantitative trait locus
SD	standard deviation
SE	standard error
SINE	short interspersed element
USDA	United States Department of Agriculture